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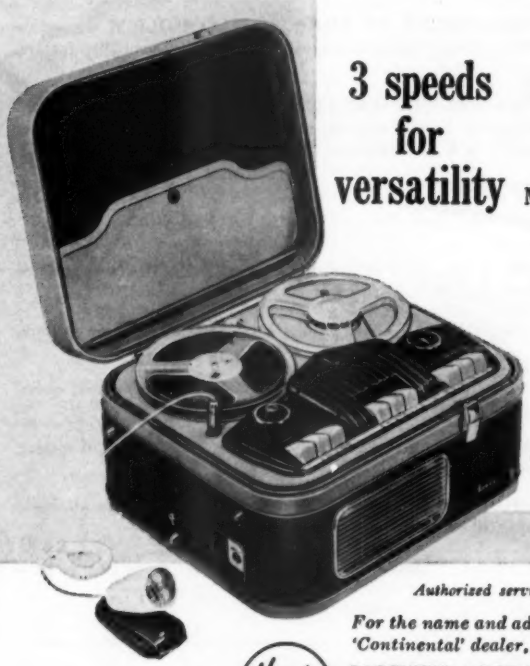

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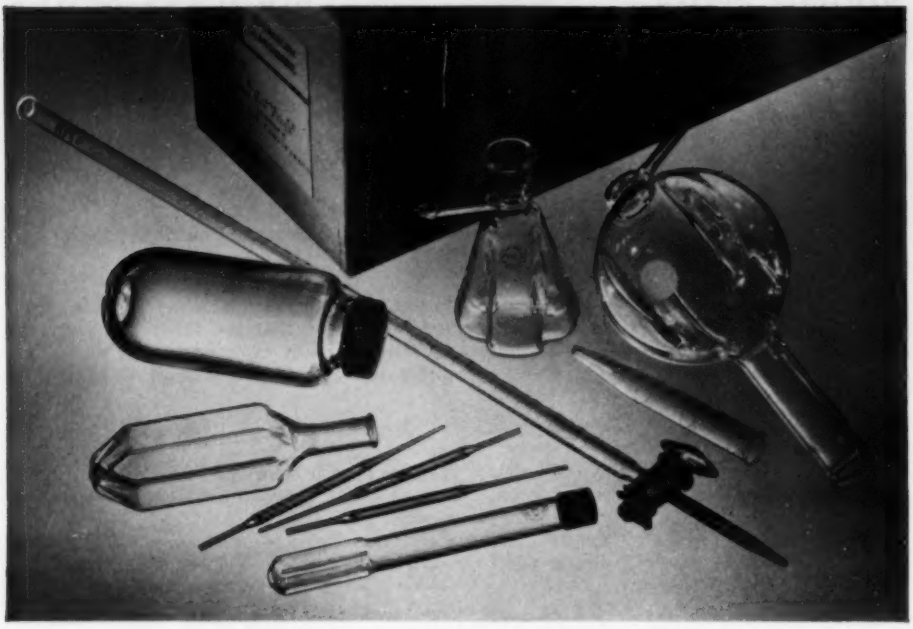

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Hypervitaminosis A and Hyperparathyroidism

Comparative Study of the Effects of Vitamin A and Parathyroid on Bone in Vivo and in Vitro, with Special Reference to Parathyroid Glands in Hypervitaminosis A

LIEUT. COL. CHARLES C. BERDJIS (MC), U. S. Army

Introduction

It has been reported for many years that an excessive intake of vitamin A can have toxic effects on laboratory animals. Hypervitaminosis A, described by several authors, creates a complex syndrome in the body, in which the first target appears to be the skeletal system. Congenital malformations, including gross anomalies in the development of the skull (Cohlan [1]) and retardation in the normal growth (Berdjis [2]), have also been described in young rats whose mothers had received excessive amounts of vitamin A during pregnancy.

Although excessive doses of vitamin A cause severe changes in the skeleton, the exact mechanism of the action of vitamin A remains obscure. Fell and Mellanby,[3-5] who studied the effects of vitamin A on bone in tissue culture, came to this conclusion—Whether the changes resulting from hypervitaminosis A are due to a direct action of vitamin A on the bone or to an indirect action through the endocrine glands is uncertain. However, direct action was elicited by these authors.

In a previous study,[6] it was concluded that the hypervitaminosis A syndrome in living animals is similar to that produced by parathyroid. The purpose of this paper is to compare the direct action of parathyroid and excessive intake of vitamin A on bones both in vivo (experimental animals) and in vitro (tissue culture). It is also the purpose of this paper to study the parathyroid changes in hypervitaminotic A animals.

Submitted for publication Aug. 28, 1958.

From the Division of Pathology, Fourth U. S. Army Medical Laboratory, Fort Sam Houston, Texas.

Materials and Methods

Most of the experiments in the present investigation were carried out with the use of rats, and the results described below were obtained with administration of excess vitamin A both in vivo and in tissue culture. In order to compare the effects of hypervitaminosis A and parathyroid hormone, an experimental hyperparathyroidism was produced in a series of rats and in the culture medium.

A solution of crystalline vitamin A alcohol in corn oil, having a potency of 500,000 I. U. per gram, was used to feed the animals orally, at a level of 100-300 I. U. per gram of body weight per day, in addition to the standard laboratory diet.

The detailed distribution of the animals used in these experiments was as follows:

In Vivo.—Ninety-five young rats, averaging 35 days of age, were used in the following experiments: (*a*) 40 controls, untreated and fed with the standard laboratory diet; (*b*) 40 rats, fed with excessive doses of vitamin A, as indicated above, in addition to the standard laboratory diet, and (*c*) 15 rats, given subcutaneous injections with 250 U. S. P. units of parathyroid, daily for three days.

In Vitro (Tissue Culture).—In these experiments, isolated long bone rudiments (tibia) of rat fetuses at birth were used. Twenty-five newborn

rats were killed under complete asepsis, and the tibiae were isolated for tissue culture. All of the material and instruments used were carefully sterilized to avoid secondary contamination.

(*a*) Explants: Twenty-five pairs of tibiae were isolated; soft tissue was removed, cleaned, and explanted, in an equal number, as follows: One of each pair of tibiae of the same animal was explanted in normal medium (see below), and the other in the same medium to which had been added one of these two solutions: 2,000 I. U. of vitamin A per 100 ml. and 300 U. S. P. units of parathyroid per 100 ml. The tibiae were fixed onto coverslips by a clot of chick embryo extract and chick plasma, about one drop of each. This was found sufficient to keep the tibiae fixed to the coverslips in the liquid medium for the duration of the experiment.

(*b*) Culture Medium: In order to prepare a complete medium, the following composition, a modification of Eagle medium* was used:

Solution	Concentration	Amount Required for 100 Ml., Cc.
Earle's BSS†	1×	35
Chick serum	1×	15
Eagle amino acid	5×	20
Eagle vitamins and glutamine	100×	1
Distilled water	Glass-distilled twice	25.5
$NaHCO_3$	4.4%	1.5
Penicillin	100,000 units/ml.	0.5
Streptomycin	100,000 units/ml.	0.5
Nystatin (Mycostatin)	5,000 units/ml.	1.0

To the complete medium (a liquid) glucose was added to bring the concentration of glucose up to 0.16%.

The phenol red was included in the media as a color indicator of the pH. The pH ranged between 7.2 and 7.4.

The liquid medium was chosen because it was felt that the feeding and maintenance of the bone cultures could be more easily handled in a liquid medium than in a solid medium, i. e., the clot medium.

Vitamin A Preparation.—The stock contains 1,500,000 I. U. of vitamin A alcohol crystalline per gram. One gram of this crystalline vitamin A was dissolved in 15 cc. of absolute alcohol, then diluted by means of normal medium to 2,000 I. U/100 cc., as required for this experiment. Alcohol concentration of the media was 0.2% and believed not to be harmful for tissue culture.

Parathyroid Preparation.—The stock contains 100 U. S. P. units per cubic centimeter. Three U. S. P. units per cubic centimeter was added to the normal medium so that the required concentration was 300 U. S. P. units per 100 cc.

Histological Technique.—All of the animals (Table 1) were killed. The entire bony system, including femur, tibia, humerus, vertebrae, jaws, skull, ribs, and parathyroid glands, was fixed in 10% formalin, embedded in paraffin, and sectioned approximately 6μ thick.

All bones, including the long bone rudiments (tibiae) from the tissue culture at 11 days, were first decalcified, then embedded in paraffin. All tissues were stained routinely by hematoxylin and eosin, unless special mention is made.

Results

The animals were killed, as scheduled in Table 1, at varying intervals (every week)

TABLE 1.—*Schedule of Killing*

Animals	Week 1	2	3	4
Hypervitaminotic-A rats	10	10	10	10
Rats given parathyroid injections	--	--	15	--
Control rats	10	10	10	10

during experiments. For each group, records were taken of bony changes and parathyroid modification. The following data were obtained in gross and microscopic examination.

In Vivo.—Control Groups: The entire skeletal system and parathyroid glands of the control animals were examined, both grossly and histologically, and they showed no alterations.

Hypervitaminotic A Animals: Bones. Because of frequent and extensive hemorrhages and multiple spontaneous fractures in the first two weeks of experiments, the histologic pictures were somewhat confusing, and fibro-osteoclasia could not be described and isolated easily. Nevertheless, in the living animals, hypervitaminosis A caused severe changes in the skeleton, which are summarized here. The maturation and degeneration of the cartilage cells and the

* Eagle, H. J.: *J. Exper. Med.* 102:595, 1955. Mr. Adrian Rake of the Virology Division, Fourth U. S. Army Medical Laboratory, helped in conducting the tissue culture for these experiments.

† A buffered isotonic balanced salt solution.

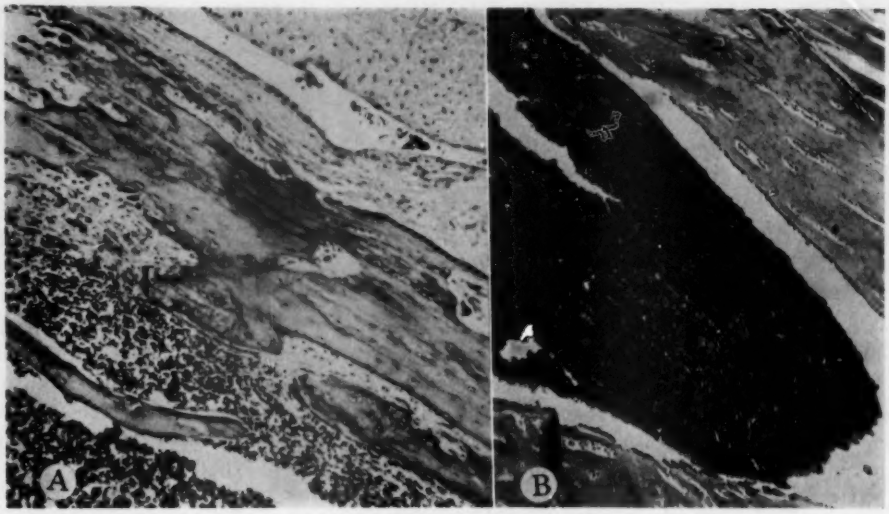


Fig. 1.—*A*, section of a long bone shaft of hypervitaminotic A rat, showing subperiosteal and subcortical lacunar fibro-osteoclasia. *B*, the same bone of a normal untreated rat. Hematoxylin and eosin; reduced 10% from mag. × 80.

replacement of cartilage by bone were greatly accelerated and resulted in the abnormally early closure of the epiphyses (Wolbach and Bessey[7,8]); rarefaction and reduced deposition of bone in the mandible (Irving[9]); retardation of osteogenesis, reduction of endochondral ossification, and a variable degree of osteoclasis in the region of the fractures (Strauss[10]) and elsewhere (Berdjis and Rinehart[6]). The fractures appeared to be due to the extensive loss of previously formed cortical bone before the newly deposited bone had acquired firmness sufficient to meet mechanical requirements (Wolbach[8]).

In the course of the third and fourth week after vitamin A administration, the skeleton underwent lacunar resorption con-

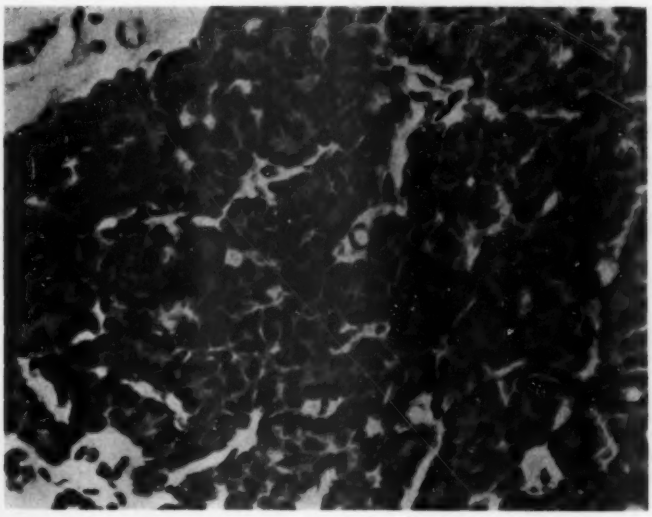


Fig. 2.—Section of a parathyroid gland in a normal untreated rat. Note the trabecular structure and two kinds of cells (rounded and elongated), equally distributed throughout the parenchyma. Hematoxylin and eosin; reduced 5% from mag. × 450.

TABLE 2.—*Parathyroid Size and Animal Weight in Normal and Hypervitaminotic-A Rats at Fifty-Five Days of Age*

Observations	Animal, No.	Animal Weight, Gm.		Parathyroid Glands			
		Range	Average	No.	Size μ		Ratio, Size / Weight
					Range	Average	
Hypervitaminosis A	10	35–60	50	20	180–435	300	$\frac{300}{50}=6$
Control	10	125–135	130	20	330–480	400	$\frac{400}{130}=3$

sistent with the early stage of generalized fibro-osteoclasia (Berdjis and Rinehart [6]).

Briefly, the total amount of bone matrix was reduced, and small, narrow, irregular, and delicate bony trabeculae were frequently encountered. About the trabeculae, and subcortical or subperiosteal regions as well, some fibrotic tissue, more or less rich in osteoclasts, indicating lacunar resorption, was present (Fig. 1). This lacunal resorption was more marked in the mandibular bone, femur, and vertebrae.

Parathyroids in Normal Rat (Fig. 2): Under normal conditions, a parathyroid gland is composed of closely packed trabeculae, lying in one or more relatively compact lobules and generally surrounded by a thin capsule. A tiny network of well-vascularized fibroconnective tissue, generally devoid of fat cells, forms the stroma. The parenchyma consists of two kinds of cells, rounded and elongated, which are usually equally distributed throughout the parenchyma. The nuclei are centrally placed, and the cytoplasm, usually ill-defined, is more or less acidophilic, without real boundaries. This histologic structure applies generally for both rat and mouse parathyroids.

Parathyroids in Hypervitaminotic-A Rat: In order to evaluate the size of the parathyroid glands, serial sections of each parathyroid were performed. Table 2 shows the average size of the parathyroids in normal and hypervitaminotic A rats. According to this Table, the parathyroid glands showed a relative increase in size in hypervitaminotic A animals. They were also moderately

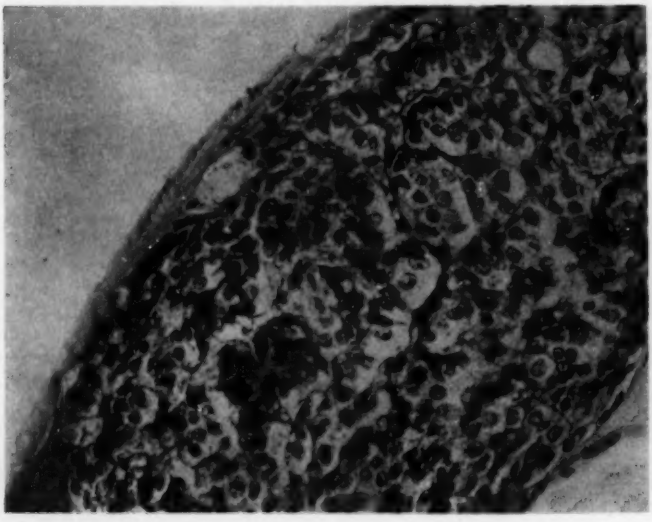


Fig. 3.—A rat parathyroid gland three weeks after excessive administration of vitamin A. This photomicrograph shows closely packed round cells with clear cytoplasm and rounded nuclei. The number of elongated cells is markedly decreased. Hematoxylin and eosin; reduced about 10% from mag. × 450.

modified in histologic structure. The parathyroid glands become more compact, losing partially or entirely their trabecular architecture. Consequently, the stroma is scanty. The cells undergo two major changes: (*a*) The rounded or polyhedral cells predominate, replacing the elongated cells, and (*b*) the cytoplasm becomes spongy and clear, losing the major part of its acidophilia (Fig. 3).

Parathyroid Hormone: The rats that received parathyroid injections showed the classical picture of experimental fibro-osteoclasia, namely, lacunar resorption of the trabecular and subcortical regions, osteoclasts in the resorptive areas, and metastatic calcification (kidney, etc.). These findings were similar to those found in hypervitaminotic A animals by Berdjis and Rinehart.[6]

In Vitro (Tissue Culture).—When we take into consideration the above findings of in vivo hypervitaminosis A and experimental hyperparathyroidism with parathyroid hormone, it is interesting, first of all, to see whether the same would occur under the recent experimental conditions in vitro. The following data are obtained in vitro in order to compare the two categories of findings:

Explants in Normal Medium (Controls): The tibiae explanted in normal medium for 11 days grew in a normal way. The cartilage and the bone matrix were differentiated as in vivo. The diaphysis contained normal bone marrow but slightly reduced in amounts, and the cartilage ends grew with a normal relationship to the metaphysis. The periosteal bone was firm and formed a well-developed coat for the marrow cavity (Fig. 4). Comparing these explants with the normal bones of living animals at the same age, it appears that the rate of growth is not modified in the tissue culture.

Fig. 4. — This photomicrograph shows the tibia of a rat fetus, explanted in a normal medium. There is a normal growth of bone and cartilage. Hematoxylin and eosin; × 6.

Fig. 5.—Opposite tibia of the animal shown in Figure 4, explanted in a hypervitaminotic A medium. There are constriction, shrinkage, and cartilage atrophy. Hematoxylin and eosin; × 6.

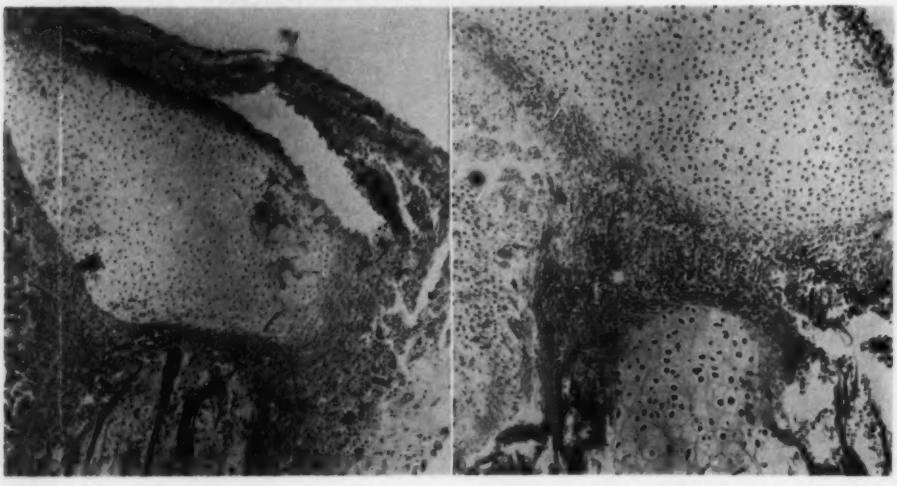
Explants in Hypervitaminotic-A Medium: The rudiments grew normally for the first two to three days, then declined during the eight remaining days.‡ In general, the rate of elongation diminished, and the tibiae became shorter and more slender than in the controls. A few explants stopped growing and began to shrink. The terminal cartilage had resorbed, atrophied, and almost disappeared (Fig. 5). The diaphyseal bone underwent excessive resorption. There were several areas of diffuse or localized fibrosis in the subcortical and subperiosteal region. A few slender trabeculae were found in the metaphysis, and they were fragile, fragmented, and more or less resorbed, disclosing varying numbers of osteoclasts.

This resorption led to constriction, and spontaneous fractures (Figs. 6, 7) generally occurred at the terminal part of the tibia. This constriction appeared in the zone of flattened cartilage cells (proliferating zone) just where the sheath of periosteal bone ended. This change was most conspicuous at the proximal end of the tibia. In some cases, however, the constriction appeared also at the distal end.

Explants in Medium with Parathyroid: Growth progressed normally at first, then declined. Parathyroid, however, did not stop entirely the normal growth of bone tibiae and did not affect the cartilage significantly. The rudiments underwent resorption; they became slender and shrunk-

‡ All explants, regardless of the medium in which they grew, remained under observation in tissue culture for a period of 10 to 11 days.

Fig. 6.—Constriction of the cartilage and cartilage atrophy in higher magnification. The cartilage cells are compressed, and some are degenerating. Hematoxylin and eosin; × 80.



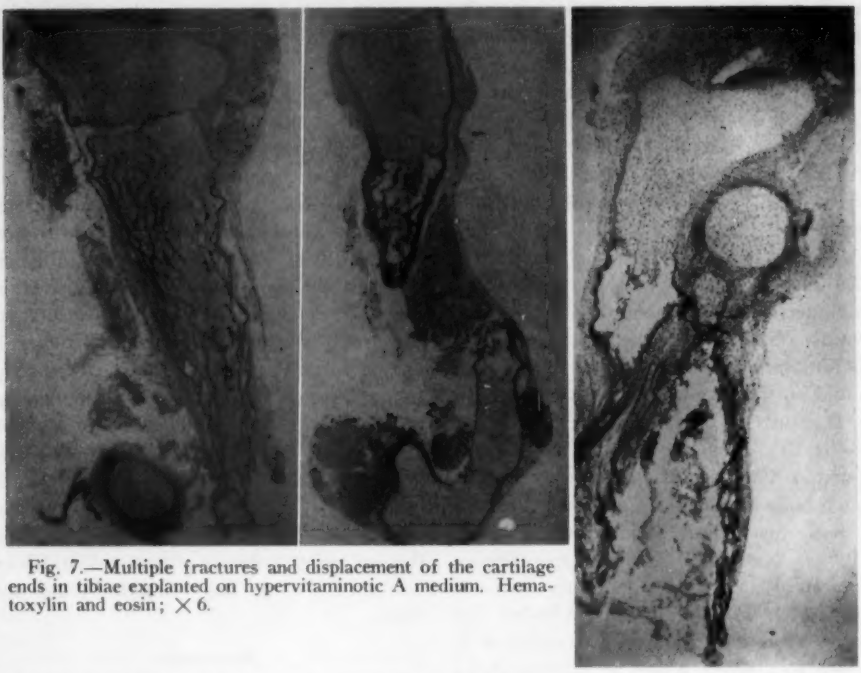


Fig. 7.—Multiple fractures and displacement of the cartilage ends in tibiae explanted on hypervitaminotic A medium. Hematoxylin and eosin; × 6.

en. The terminal cartilage grew normally in both distal and proximal ends. No constriction or spontaneous fractures were observed. The osteoclasts here, as in the hypervitaminosis A medium, were present in subcortical and subperiosteal regions and around the slender trabeculae.

Comment

In a previous study, Berdjis and Rinehart [6] pointed out that the effects of administration of excessive vitamin A on bone are comparable to those produced in rat by parathyroid hormone. In the present investigation, this study was extended in tissue culture and the changes were produced in bone both by vitamin A and parathyroid hormone.

From the analysis of histologic findings and the data summarized in Table 3, it seems that the vitamin A and parathyroid

TABLE 3.—*Comparative Study Between the Effect of Vitamin A and Parathyroid on Bone in Vivo and in Vitro*

	Living Animals (In Vivo)			Tissue Culture (In Vitro)		
Observations (Long Bones)	Vitamin A	Parathyroid	Control	Vitamin A	Parathyroid	Control
Size	Reduced	Reduced	Normal	Reduced	Reduced	Increased
Linear growth	Arrested	Reduced	Normal	Arrested	Reduced	Normal
Terminal cartilage	Disturbed	Almost normal	Normal	Atrophied	Almost normal	Normal
Differentiation of chondroblasts	Moderately accelerated	Normal	Normal	Not arrested	Normal	Normal
Maturation and calcification	Failure	Disturbed	Normal	Failure	Disturbed	Normal
Matrix formation	Reduced	Reduced	Normal	Dissolution	Reduced	Normal
Spontaneous fractures	Frequent	?	None	Frequent	?	None
Periosteal bone & shaft	Resorbed	Resorbed	Normal	Resorbed	Resorbed	Normal
Osteoclasia	Present	Present	Normal	Present	Present	Normal
Soft tissue surrounding bone	Normal growth	Normal growth	Normal growth	Normal growth	Normal growth	Normal growth

have a similar action on bones, namely, resorption and subsequent lacunar fibro-osteoclasia. Cartilage, however, reacts differently; while cartilage is greatly affected by heavy doses of vitamin A, the parathyroid seems to have no significant action on cartilage. Endochondral ossification, however, is highly reduced or arrested in both instances.

Although excessive doses of vitamin A cause severe changes in the skeleton, the exact mechanism of action of vitamin A remains obscure. In the living animals, hypervitaminosis A renders the bones fragile, and so they sometimes suffer spontaneous fractures. The fractures appear to be due to the extensive loss of previously formed cortical bone before the newly deposited bone has acquired firmness sufficient to meet mechanical requirements (Wolbach [8]). Nevertheless, the spontaneous fractures also occurred frequently in tissue culture rich in vitamin A. Whether the changes resulting from hypervitaminosis A are due to a direct action of vitamin A on the bone or to an indirect action through the endocrine glands is uncertain.[3-5,13]

Barnicott, in 1948,[12] showed direct local action of vitamin A on bone by implanting the bone fragments in the cerebral hemispheres of mice, in which he introduced the crystals of pure vitamin A. It is well known that the parathyroid fragments introduced into the skull, behind the parietal bone, reproduce the same effect, namely, resorption of bone and sometimes perforation.

Fell and Mellanby,[3-5] in a series of experiments, studied the direct action of vitamin A on bone by demonstrating both disintegration of cartilage matrix and resorption of bone in fetal mouse bones grown in tissue culture. Our own observations are in close agreement with the findings of these authors.

Berdjis and Rinehart [6] described frequent lacunar bony resorption in hypervitaminosis A animals, indicating direct action of vitamin A on bone in living animals. These authors also elicited a similarity of action on bone between vitamin A and parathyroid. In this study we confirmed previous reports [2,6,13] that endocrine glands, except parathyroid, have no part in hypervitaminosis A syndrome. The parathyroid glands, however, showed evidence of increasing volume and predominance of rounded cells with more compact parenchyma, interpreted as slight hyperplasia. It is more tentative than conclusive to say that the parathyroid changes are responsible for bone alteration. It may be suggested, according to a statement of Askanazy and Erdheim (Wernly and Berdjis [11]), that this slight parathyroid hyperplasia is the response of bone change rather than a cause. In the course of these experiments we found a close similarity between vitamin A and parathyroid action on bone in living animals and in tissue culture as well (Table 3). This implies that the interrelationship of vitamin A and bone is comparable to that of parathyroid and bone.

Summary

Hypervitaminosis A and hyperparathyroidism are produced in young rats, which are killed at regular intervals with the same number of control animals, as indicated in Table 1. Bones and parathyroid glands are systematically studied. In order to compare these findings with those produced in vitro, the tibiae of rat fetuses at birth are explanted in normal medium, hypervitaminotic A medium, and parathyroid medium for a period of 11 days.

Experimental hypervitaminosis A appears to be somewhat similar to experimental hyperparathyroidism both in living animals and in tissue culture.

The parathyroid glands in hypervitaminotic A animals undergo modification consistent with mild hyperplasia, which is believed to be due to bone alterations (fibro-osteoclasia).[6,11]

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The Pathogenesis of Bone and Joint Infection Produced in Rats by Streptobacillus Moniliformis

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A reproducible infection of bone and joint in rats has been demonstrated in high incidence with Streptobacillus moniliformis,[1] a micro-organism usually considered to be a commensal or of low virulence for this host.[2,3] A strain recently isolated in this laboratory from a middle ear infection of a rat has been found to have a specific affinity for joints, bone, and periarticular tissue when injected intravenously. To date, gross and/or microscopic lesions have developed in 93% of more than 100 rats infected experimentally with this micro-organism. In animals showing acute changes, the infecting micro-organism has been recovered from 19 of 21 wrists or ankles from which material was cultured during the acute, 5- to 11-day stage. Blood cultures made during this same period have been positive in only 1 of 15 animals.

The present report deals with the evolution of the acute lesions in joints and adjacent tissues from the time of inoculation. Periodic histological examinations of joints and bones of a series of infected animals were undertaken, and simultaneous bacteriological studies of these tissues and of blood were made. The pathogenesis of the acute lesions and their possible correlation with the presence of the micro-organism are described.

Materials and Methods

Previous techniques were employed to produce lesions of joint regions in rats.[1] In brief, young Holtzmann-Fisher cross rats, 2½-3½ months old, raised so as to be free of infection, were given intravenous injections (tail vein) with 2.0 ml. of a 20- to 22-hour broth culture of S. moniliformis. The culture was a fresh animal passage of Strain "C," obtained by culturing material from the joint region of an experimentally infected rat and carried in beef-infusion-ascitic-fluid broth medium. Nineteen animals were given injections, and two animals were killed every 24 hours (±2 hours) for seven days. The remaining five rats were kept for determination of the incidence of gross lesions in this experiment (Experiment 49). Ten of eleven rats showed gross changes involving one or more joints five to seven days after being given injections.

Animals were killed with ether at the specified intervals, and both wrists were arbitrarily taken for examination, whether or not they showed gross changes (these changes first appeared on Day 5 after injection). The right forepaw, including the wrist, was selected for histological study. The tissues were fixed in 10% buffered formalin, decalcified in 5% formic acid, and embedded in paraffin. Serial sections 5μ thick were made in a sagittal plane; each 25th section was stained routinely with hematoxylin and eosin. In some sections where inflammation was found, buffered Giemsa's (at pH 2.75 and 3.62) and Steiner's stains were also applied. Animals from earlier experiments, killed at 17 days and 33 days post injection, were used for the description of the older lesions.

Under aseptic conditions, 3-5 ml. of blood was drawn from the inferior vena cava for culture, and the left forepaw was removed for culture. The skin covering the left wrist was sterilized with Crystamine* and acetone and removed, and the wrist was split and ground in a mortar and pestle with sterile sand and 2.0 ml. of sterile 0.85% NaCl. Each blood sample was plated on two rabbit-blood agar plates (0.2 ml. each), and the remainder was divided between two tubes of beef-infusion broth (at pH 7.6-7.8) enriched with 20% human ascitic fluid. Each saline emulsion of joint was plated on rabbit-blood agar (0.2 ml.); approxi-

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* Crystamine is trade name (of Crystal Soap Chemical Co., Inc., Philadelphia) for a common type quarternary ammonium disinfectant. Used here at 800-1,000 ppm. quarternary compound. Active ingredient is alkyl (C_8 to C_{18}) tolyl methyl trimethyl ammonium chlorides.

TABLE 1.—*Inflammatory Changes and Cultural Recovery Following Intravenous Injection of S. Monilformis (Experiment 49)*

Rat No.	Days Post Inoc.	Source of Cultural Recovery		Microscopic Inflammation in Right Forepaw					Gross Changes			
									Wrist		Ankle	
		Blood	Left Wrist	Metaphysis	Periosteal Ring	Periarticular Tissue	Synovial Tissue	Diaphysis or Small Bone	Left	Right	Left	Right
1	1	+	+	+	+	0	0	0	0	0	0	0
3	1	+	+	0	+	+	0	0	0	0	0	0
4	2	+	+	0	0	0	+	0	0	0	0	0
5	2	+	+	+	+	+	+	0	0	0	0	0
6	3	0	+	0	0	0	0	0	0	0	0	0
7	3	+	0	0	0	0	0	0	0	0	0	0
8	4	+	0	0	0	0	0	0	0	0	0	0
9	4	0	0	+	0	+	0	0	0	0	0	0
10	5	0	0	0	0	0	0	0	0	0	0	0
11	5	0	0	+	0	0	0	+	0	0	+	0
12	6	+	+	N E *	N E	N E	N E	N E	+	+	+	0
13	6	0	+	0	0	0	0	0	+	+	0	0
14	7	0	0	+	0	0	0	0	+	+	0	0
15	7	0	0	0	0	0	0	+	+	0	+	0

* N E indicates not examined.

mately 1 ml. was placed in beef-infusion-ascitic-fluid broth (pH 7.6-7.8), and 0.2 ml. was taken immediately from this broth culture and plated on rabbit-blood agar. Thus, four primary cultures of blood and three of joint were made for each animal. Subcultures were made whenever indicated to establish the presence of growth or the identity of the micro-organism. All cultures were inoculated at 37 C and examined daily for at least one week.

Results

A. Bacteriological.—The cultural recovery of S. moniliformis from rats given intravenous injections is recorded in Table 1. All blood cultures were positive at 24 and 48 hours after injection. Blood cultures were positive in one of two rats at 72 hours and one of two rats at 96 hours post injection. Cultures were negative on Days 5, 6, and 7, with the exception of one positive culture on Day 6.

All wrist cultures were positive at 24 and 48 hours after injection. Cultures of wrist region were positive in one of two rats at 72 hours and were negative on Days 4 and 5. Wrist cultures were positive in both rats on Day 6 and negative in both rats on Day 7.

B. Gross Changes.—The occurrence and location of gross changes in the present series are recorded in Table 1. Changes observed in all animals infected with S. moniliformis for various purposes in several experiments are recorded in Table 2. These changes, occurring in the regions of the large joints of the extremities, have been noted as swelling, redness, or manifest tenderness and stiffness. Any one of the above signs has occurred singly or in combination. Joint-region involvement has been single or multiple, at times affecting all four extremities simultaneously. Occasionally it has been transitory and migratory in character, appearing first at one joint, regressing, and then appearing at another, at intervals ranging from 12 hours to 3 days.

Fig. 1.—Swelling of right wrist, six days after intravenous injection of S. moniliformis.

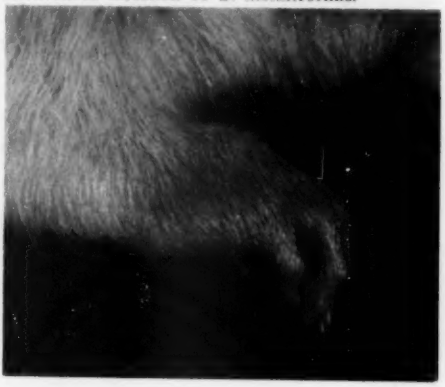

TABLE 2.—*Summary of Gross Changes in Rats Following Intravenous Injection of S. Moniliformis*

Experiment No.	Rats Infected, No.	Rats Lesions, No.	Regions Involved					
			Wrist		Ankle			
			Left	Right	Left	Right	Other	Both Wrists
28	5	5	5	2	0	1	R. Hip-1	2
29A	5	5	1	3	4	5		0
29B	16	13	4	5	7	9		2
30	6	6	6	6	3	0	R. Hip-1	6
31	8	8	3	6	2	5		2
40	8	8	7	7	3	1	L. Hip-1	6
48	10	9	4	6	3	3		3
49	11	10	6	4	4	4		3
50	35	33	21	28	12	11	L. Hip-1 R. Hip-1	18
53	12	12	11	9	2	4	R. Hip-1	8
Total	116	109 (93.8%)	68 (58.7%)	76 (65.5%)	40 (34.5%)	43 (37.1%)	6 (5.2%)	50 (43.1%)

The assessment of these gross changes has limited accuracy, particularly when they are minimal. Occasionally, extensive changes in large joints have been found histologically but not grossly. The frequency of gross lesions has been greatest on the fifth to seventh day after intravenous injection, less on Day 4, and least on Day 3. Figure 1 shows an involved wrist at the sixth day after injection.

The incidence of gross lesions has been greatest in wrists, next in ankles, and least in hips (Table 2). There was a nearly equal extent of involvement of left and right sides, of wrists (58.7% and 65.5%), and of ankles (34.4% and 37.1%). In animals showing any wrist changes, 53.2% had simultaneous involvement of both wrists.

C. Histological.—The occurrence and location of histological changes are recorded in Table 1. A minute and focal acute inflammatory reaction was found in the forepaw within 24 hours after injection. The inflammatory cells were of several types, predominantly small mononuclear cells and a variable proportion of polymorphonuclear

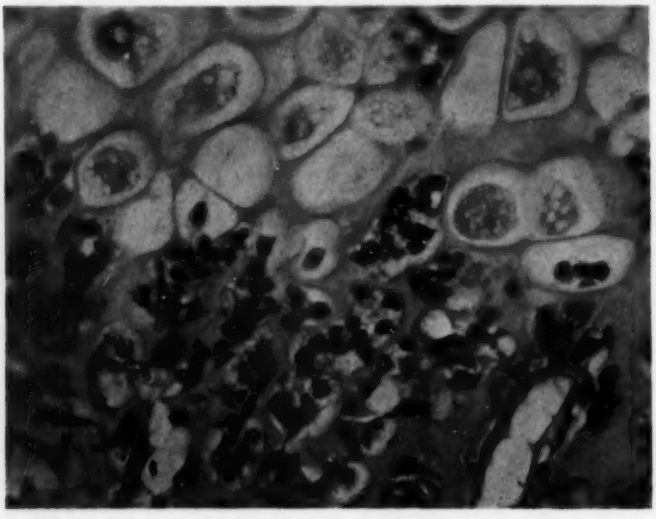

Fig. 2.—Acute inflammatory reaction in metaphysis, 24 hours after injection. Polymorphonuclear neutrophils infiltrate the subchondral marrow spaces as well as the lacunae of the degenerating cartilage columns of the epiphysis. Hematoxylin and eosin; reduced 10% from mag. × 530.

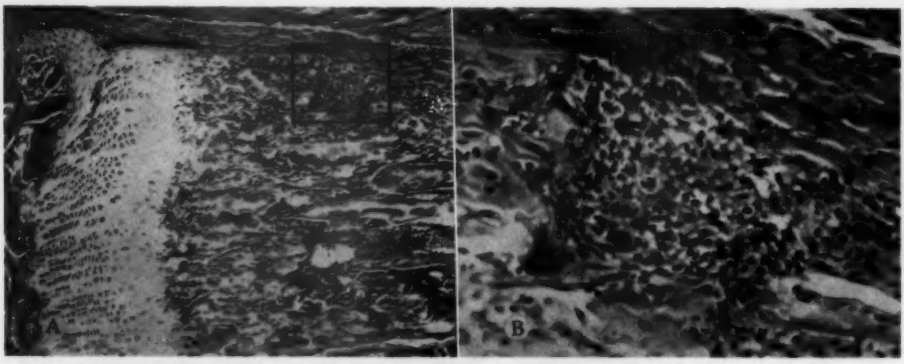

Fig. 3.—Acute inflammatory reaction at periosteal ring, 48 hours after injection. *A*, a minute inflammatory infiltrate is present in the discontinuous cortex immediately proximal to the epiphyseal plate. Hematoxylin and eosin; reduced 40% from mag. × 110. *B*, higher magnification of framed area in preceding. Polymorphonuclear neutrophils and mononuclear cells comprise the infiltrate; reduced 40% from mag. × 385.

neutrophils. The location of the lesions in the forepaw tissues varied; initially the most conspicuous reaction was in the radial metaphysis, especially on the flexor aspect. Many of the marrow spaces in the spongiosa layer and lacunae of the hypertrophic cartilage columns contained extravasated polymorphonuclear neutrophils (Fig. 2). Minute abscesses formed in 24 or 48 hours at the periosteal ring, that is, the periosteal margin of the growth zone in which there is a physiological discontinuity of the bone cortex (Fig. 3). In several instances, the inflammatory reaction was seen to extend through this breach into adjacent fibroadipose and periarticular structures (Fig. 4). Synovitis was observed in two animals, in one of which there was no observed bone or periosteal involvement. In view of the fact that the series of sections was incomplete, it is conceivable that even here a site of bone involvement had not been included in the section. Micro-organisms could not be identified with certainty in the lesions when special staining techniques were used.

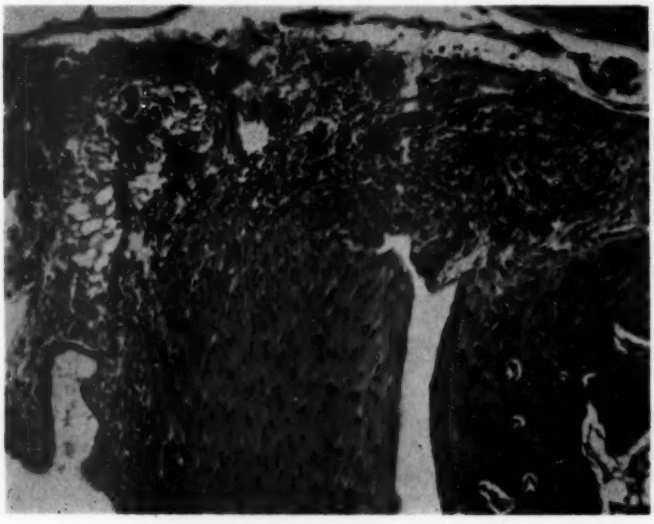

Fig. 4. — Inflammatory reaction in periarticular tissues of wrist 24 hours after injection. Hematoxylin and eosin; reduced 10% from mag. × 128.

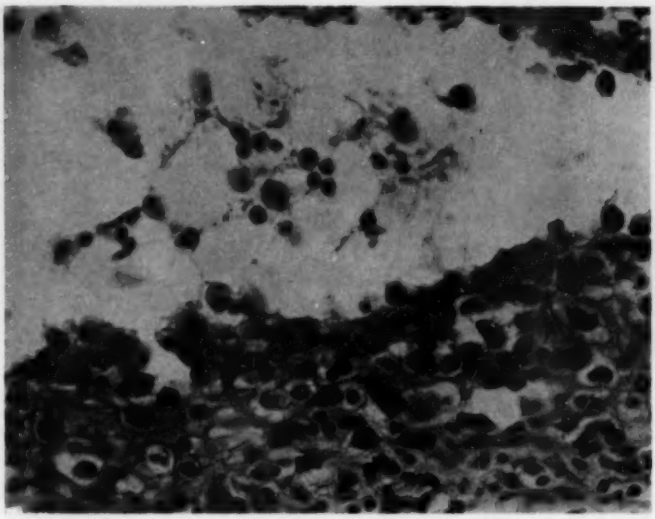

Fig. 5.—Subacute synovitis, 17 days after injection. Synovial lining cells are swollen and hyperplastic. Polymorphonuclear neutrophils and mononuclear cells infiltrate the synovial lining. Joint space contains similar inflammatory cells and synovial lining cells. Hematoxylin and eosin; reduced 10% from mag. × 460.

Older lesions showed consistent involvement of bone. This was seen at 6 and 7 days in the present series and in the animals examined at 17 days and 33 days from other experiments. A defect in the cortex through which the osseous and para-articular inflammation extended was sharply defined. In some cases, continuity of the process in metaphysis and epiphysis was established through a gap in the epiphyseal plate. Occasionally, the inflammation remained confined to the medulla of the bone and did not affect the soft tissue. After the first week, the inflammatory site contained somewhat fibrous granulation tissue and inflammatory cells of predominantly mononuclear character. Bone cortex and trabeculae had disappeared in the immediate areas, but radial seams of new bone and cartilage formation were present at the adjacent perichondrium and periosteum. In the severest lesions, purulent exudate filled

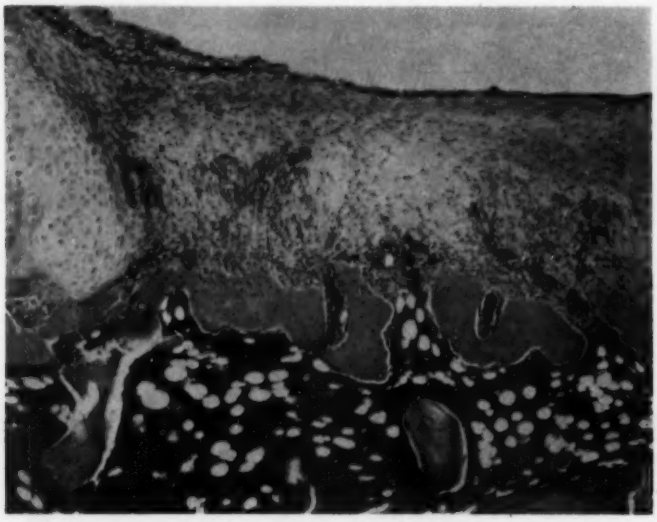

Fig. 6.—Joint destruction, head of tibia, 33 days after injection. Articular cartilage and portions of the subchondral plate are replaced by fibrous tissue and callus. The surface is lined by synovial type cells. Hematoxylin and eosin; reduced 10% from mag. × 85.

the joint spaces and the synovial lining cells were swollen and hyperplastic (Fig. 5). The articular cartilage was least affected. However, extensive destruction of articular cartilages and deformity of the epiphysis of the tibial head were found in a knee 33 days after injection. Inflammatory cells had largely disappeared. The cartilages were largely replaced by compact, synovial-like fibrous tissue, and the meniscus was bound to the tibia by a broad fibrous adhesion (Fig. 6). Lesions were not found in any organs or other soft tissues examined.

Comment

The inflammatory reaction produced by intravenous injection of S. moniliformis affects chiefly the ends of the bones and the adjacent periosteal or perichondrial tissues. In some cases, the reaction has been confined to the medullary cavity. The synovial tissues are involved infrequently in the absence of bony changes; more often there is continuity between the inflammatory processes in bone and periarticular and articular tissues. The external evidence of "arthritis"—the swelling and redness—apparently reflect the presence of inflammation in the extra-articular soft tissues. Inflammation develops within 24 hours after injection; however, it is not of sufficient magnitude to be externally evident until a few days have elapsed. The sequence of events is illustrated diagrammatically in Figure 7. Although both inflammatory changes and recovery of the micro-organism were demonstrated in the forepaw of each animal examined during the first 48 hours, such findings were inconsistant during the following 4 days.

The advent of acute microscopic inflammation on the first two days and its apparent disappearance on the third and fourth days may be a chance occurrence. The experimental series is small, and the involvement or sparing may have been due to chance of sampling a particular extremity. In other experiments cultural recovery

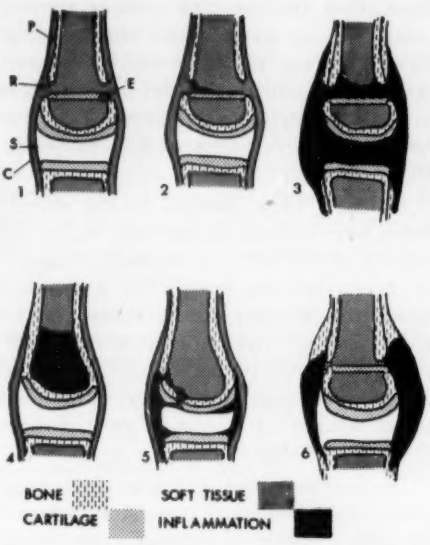

Fig. 7.—Schematic representation of several types of arthritis (1) normal diarthrosis of rat; (2) early S. moniliformis infection; (3) advanced S. moniliformis infection; (4) S. moniliformis infection confined to medulla of bone having fused epiphysis; (5) rheumatoid arthritis; (6) periarthritis; *C*, joint capsule; *E*, epiphyseal plate; *P*, periosteum; *R*, periosteal ring; *S*, synovial tissue.

of the micro-organism and histological study had always been made at optimal time after selecting the extremities with maximum gross changes. Gross changes have been noted in at least one extremity in 93% of 116 rats given injections. Observations that in 116 animals the left forepaw was grossly affected in 59% and the right forepaw in 66%, with symmetrical involvement in 53%, would indicate that the infection is rather uniformly disseminated and that arbitrary sampling of one wrist would be likely to give a representative result.

The observations of inflammatory reaction and positive cultures in the wrists during the first 48 hours in each animal were consistent with this hypothesis. The fact that inflammatory lesions and positive cultures were much less frequent during the next two to three days suggests that there was a genuine regression and that the in-

flammatory reaction and positive cultures reappeared in some joints after this interval. During this interval, the bacteria may have proliferated in sufficient numbers in some foci to provoke a second inflammatory reaction, extensive enough to be observed grossly.

Although wrist cultures in the current series were positive in only two out of six animals at the five- to seven-day stage, it has been noted that these wrists were taken arbitrarily, whether or not they showed gross changes. The recovery of the micro-organisms from 19 out of 21 animals in other experiments was from wrists and ankles selected for maximum gross changes at the 5- to 7-day stage.

The production of infectious, hematogenous arthritis in small laboratory animals has been reported many times. In careful studies of arthritis caused by streptococci and other agents in rabbits, the initial articular reaction has been described as an acute synovitis.[4,5] According to Schaffer and Bennett,[6] intravenously injected pneumococci enter the joint fluid through synovial vessels within 24 hours. A number of investigators, however, have observed suppurative involvement of the bone with extension of metaphyseal abscesses into articular tissues in hematogenous streptococcal arthritis of rabbits.[7,8]

In the present experiments, the bone rather than the synovial tissue appears to be the primary site of involvement, with secondary articular and periarticular changes. This pattern is not unique for this micro-organism or this animal. Primary bone lesions extending into the joints have been noted in staphylococcal infections of rabbits[9-11] and of ducks.[12] Although not explicitly diagnosed as such, suppurative involvement of bone is clearly described and illustrated in hematogenous arthritis of rats, caused by streptococci[13,14] as well as by corynebacteria.[15] Mice, unlike rats, are highly susceptible to infection with S. moniliformis.[16-18] When the infection is not rapidly fatal, arthritis is its most characteristic feature. The lesion is a suppurative one and destroys bone, joint, and para-articular structures. Occasionally, micro-organisms have been seen within mononuclear cells of the exudate and have been recovered in culture of the skeleton lesions after many months.

It has been suggested that the predisposition for blood-borne infections to become localized in the metaphysis depends upon the character of the circulation in this part. The capillaries here are end-vessels, and local impaction is favored by the lack of collateral vascular egress. A factor that may be operative in the localization of infection in the joint or in the metaphysis is the age of the animal. Inoculation with staphylococci produced osteomyelitis in young rabbits[11] but arthritis without osteomyelitis in adult rabbits.[19] The epiphyses of rabbits, unlike those of rats, fuse during the first year of life, and the vascular peculiarities of the metaphyseal region are no longer available for influencing bacterial localization. Although many of the epiphyses of long bones of rats remain open through life, some, including the distal tibia and fibula, close at approximately 3 months of age. This may account for the greater amount of wrist than ankle involvement in the present studies. Many types of hematogenous infection of bone and joint in humans characteristically affect younger age groups. Localization within the metaphysis occurs early during the stage of bacteremia. The joint culture is found to be positive when the infection extends into the joint, although the blood culture may be negative. Buddingh[20] observed that S. moniliformis had an affinity for synovial tissues of the chick embryo. Angevine[21] has suggested that size of inoculum may also be a factor in determining whether synovial or ossific lesions develop and that a smaller number of micro-organisms favors the former.

Thus, the term hematogenous "arthritis" is not adequate. The lesion may correctly be called an infectious osteoarthritis, but the term osteoarthritis, although accurate,

is used widely as a synonym for human degenerative joint disease. Analogies have been drawn frequently between the infectious joint lesions of experimental animals and human rheumatoid arthritis. Although subchondral granulations may be seen in rheumatoid arthritis both at the margins of the joint and subjacent to ulcerated portions of the articular cartilage, the rheumatoid lesion is predominantly a synovitis (Fig. 7). The differences between the lesions reported here and those of rheumatoid arthritis are emphasized, since recent findings indicate that sera of these infected rats have appreciable levels of abnormal euglobulin fractions similar to those found in human rheumatoid arthritis.[22] Infection of the articular regions of rats with pleuropneumonia-like organisms is often described as arthritis. However, several investigators have noted that the lesions are more characteristically located in the periarticular tissues than in the joints proper and that osteomyelitis may occur.[23,24] Such infections have been employed widely both as an experimental model of rheumatoid arthritis and for the testing of various antirheumatic compounds. Finally, these lesions should be distinguished from other types of predominantly periarticular inflammation in the paws of rats (Fig. 7), namely, those produced by plantar pad inoculation (so-called topical irritation arthritis), by decubitus ulceration and abrasion with secondary infection, and by injection of Freund's adjuvant.

Summary

The inflammatory reaction produced in rats by intravenous injection of Streptobacillus moniliformis affects the bone primarily, with secondary periarticular and articular involvement.

Acute inflammatory lesions were found, and the micro-organism was recovered from joint regions and from the blood consistently at 24 and 48 hours after injection. During the next few days, the incidence of inflammatory lesions varied, as did cultural recovery of the micro-organism from blood and joint regions.

Gross changes have been noted in 93% of a total of 116 rats infected with this micro-organism, with highest incidence in wrists and ankles. At the "acute" stage of maximum gross involvement, cultures of affected joint regions have been positive in 91% of 21 animals, while blood cultures at the same stage have been positive in only 6.7% of 15 animals.

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Bone Marrow Embolism to Lung Following Sternal Puncture

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Red marrow emboli to the lungs have been noted on occasion, particularly in fatal cases of multiple fractures. There is but a single known reference in the literature, however, which mentions that such emboli may also arise as the result of sternal puncture.[6] The following example is presented to draw further attention to this phenomenon.

Report of Case

A 70-year-old white man, with a long-standing history of chronic pyelonephritis and renal failure, died at this hospital in April, 1958. Autopsy (Dr. Gaeton Di Mattei, prosector) confirmed the clinical diagnosis. An incidental microscopic finding consisted of a single solidly lodged red marrow embolus within a small pulmonary vessel (Figure). No associated emboli, thrombi, arteritis, or infarction were noted. Chronic passive congestion and edema were the only other significant changes in five random blocks of lung tissue. Clinical records disclosed that sternal puncture and aspiration biopsy with a Turkel needle had been performed nine days before death.

Comment

The sole known reference to marrow embolism from a sternal biopsy site is a comment from the floor by Montgomery during discussion of a paper by Fisher.[5] The latter author has studied 480 histologic sections from 96 cases of fracture fatalities and reported an incidence of marrow embolism in 19.8%. Prior investigation by Lindsay and Moon had established that true hematopoietic fragments may appear in the pulmonary circulation independent of fat emboli from medullary spaces. In a carefully studied series of 27 cases, Rappaport, Raum, and Horrell concluded that trauma to those parts of the skeletal system containing red marrow is the primary etiologic factor in this condition, but such trauma need not be external. Since, as these authors conclude, sinusoidal vascular channels are separated from hematopoietic cells only by a single layer of endothelium, local pressure, sometimes due to violent muscular contracture, may prove sufficient to rupture sinusoids and sweep marrow fragments into the venous circulation. This accounts for an occasional case of marrow embolism coincident with eclampsia, tetanus, and other convulsive states.

At times marrow emboli may be sufficiently large or dispersed to cause respiratory embarrassment, but no fatalities attributable to this cause had been formally documented before 1953.[3] However, the patient recalled by Montgomery, in 1951, died six hours after sternal puncture. It becomes unnecessarily academic to attempt differentiation of a "pure" marrow embolus (normally about 50% adipose tissue) from a fat embolism that has hematopoietically inactive medullary spaces. The point in issue, marrow embolism subsequent to sternal puncture, is no longer theoretical. Such emboli may be bland and few (present case) or fatal and multiple (Montgomery's experience).

Documented instances of red marrow emboli judged to be the immediate cause of death have begun to appear only recently. These have been encountered with neoplastic and tuberculous pathologic fractures of vertebrae[1,3] and after thoracotomy.[7] A somewhat different mechanism,

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Marrow embolus lodged in branch of pulmonary artery. The myeloid elements show active hematopoiesis. Hematoxylin and eosin; × 75.

namely, bone marrow infarction, has led to fatal red marrow embolus in a few cases of sickle-cell anemia and hemoglobin C disease.[8]

Summary

The second reported example of red marrow embolism to the lungs incident to needle aspiration of a marrow space is presented. This potentially dangerous phenomenon adds another, although so far rare, risk to this common diagnostic procedure.

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Renal Lesions in Experimental Hypertension

Morphological Changes in the Kidney of Rats Rendered Chronically Hypertensive Following a Period of Choline Deficiency

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Griffith and Wade [1] first demonstrated the acute hemorrhagic renal lesion induced in rats by choline deficiency and described the dilated tubules, casts, atrophic glomeruli, and interstitial fibrosis which developed later in such animals. Hartroft and Best [2] demonstrated the occurrence of hypertension in rats that had been fed a choline-deficient diet at the time of weaning. Grollman and White [3] showed that chronic hypertension occurred regularly following a deficiency of choline or of potassium in the diet of weanling rats and found that the phenomenon was potentiated by unilateral nephrectomy, administration of ethyl alcohol, and administration of excessive sodium chloride. They utilized this procedure for maintaining a colony of chronic hypertensive animals.

In order to evaluate the factors involved in its pathogenesis, kidney tissues were examined at various stages during the development of hypertension following exposure to a low-choline diet.

Methods

Piebald rats of the Long-Evans strain reared in the laboratory were placed on the choline-deficient diet at the time of weaning for periods of approximately two weeks. The operative procedures, diet, determination of blood pressure, etc., were carried out as described previously (Grollman and White [3]). The kidneys, removed under ether anesthesia, and other tissues were examined at intervals of a few months to a year after exposure to the diet. Tissues were fixed in 10% formalin or cold neutral buffered formalin, stained with hematoxylin and eosin, oil red O, Mallory's aniline blue, Van Gieson's, and periodic acid-Schiff-Alcian blue stains.

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Observations

The changes observed in the kidney are summarized in the Table. Immediately after the rats were subjected to the choline-deficient diet, only slight changes, characterized by fatty degeneration of the proximal convoluted tubules and a few casts in the distal convoluted and collecting tubules, were noted. There was slight swelling of the cells lining the lower loops of the proximal convoluted tubules. At this stage, definite glomerular abnormalities could not be detected by the standard methods of morphological examination; nor were the characteristic hemorrhagic renal lesions observed, presumably because of the relatively short period of dietary deficiency.

Renal Changes Prior to Hypertensive Stage.—Alterations in the glomeruli were observed as early as two weeks after exposure to the dietary deficiency. These changes (Fig. 2; Fig. 1 is a normal glomerulus for comparison.) consisted of thickening of the glomerular basement membrane, an increase in the number and swelling of the glomerular epithelial cells, and clumping of epithelial cells between the capillary loops. These changes increased progressively during the subsequent several months, during which period the renal tubules showed minor or no changes. The glomerular lesions were demonstrable most clearly with periodic acid-Schiff (PAS) stains or with PAS-Alcian blue, which revealed a strongly PAS-positive membrane that became progressively thicker and the appearance of a less intensely PAS- or Alcian blue-stainable

Evolution of Various Changes in Kidneys of Rats After a Period of Choline Deficiency at Time of Weaning

Time Elapsing After Subjection to Choline Deficiency	Animals, No.	Glomeruli	Juxtaglomerular Cells	Hyaline & PAS-Positive Droplets in Proximal Convoluted Tubular Cells	PAS-Positive Material (Mucopolysaccharide) in Proximal Convoluted Tubular Lumens	Casts & Dilatation of Tubules	Arterioles	Average Arterial Blood Pressure Mm. Hg.
1 day-1 mo.	19	Increased cellularity & swollen epithelial cells	Normal granulation	Slight increase	Slight increase	None	Normal	120
1-6 mo.	16	Increased thickness of basement membrane	Normal granulation	Slight increase	Slight increase	None	Normal	130
6 mo.-1 yr.	10	Further increased thickness of basement membrane; narrowed capillaries	Normal to slight decrease in granules	Moderate increase	Moderate increase	Slight to moderate	Moderate arteriolar sclerosis & dilatation of afferent arterioles inside glomeruli	150
1 yr. & over	23	Marked thickness of basement membrane & fibrosis, with marked reduction of capillary beds	Marked reduction or absence of granules	Moderate to marked increase	Moderate increase	Moderate to marked	Marked arteriolar sclerosis & dilatation of precapillary arteriole in glomeruli	170

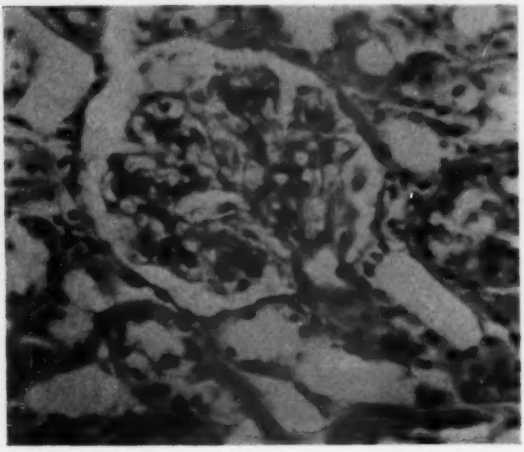


Fig. 1.—Normal glomerulus from young adult rat. PAS stain; × 350.

material around the basement membrane (Fig. 3).

During the first few months after choline deficiency the proximal convoluted tubules contained PAS-positive and proteinaceous material (positive for reactive NH_2 groups) in their lumina in greater amount than is found in normal animals. The cells of the proximal convoluted tubules contained a definite increase in eosinophilic, PAS-positive material in the form of rounded droplets (Fig. 4), as compared with normals. These droplets were indistinguishable from the hyaline droplets of renal tubular cells seen in subjects with proteinuria.[4]

The periodic acid-Schiff stain was utilized to study the granules of the cells of the

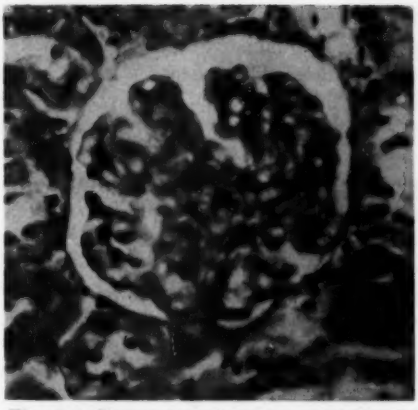


Fig. 2.—Glomerulus of rat one month after period of choline deficiency. There is thickening of the basement membrane and of the glomerular capillary walls. PAS stain; × 350.

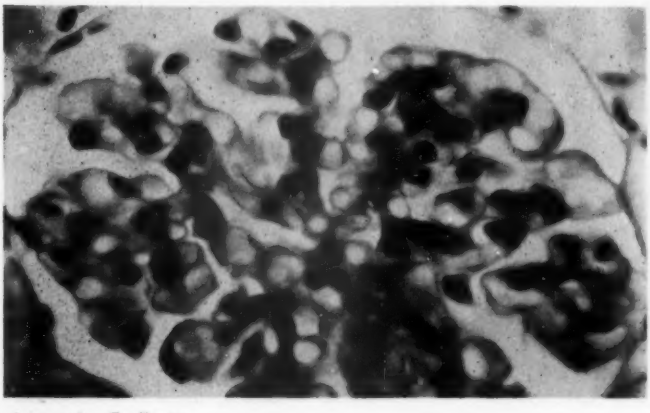


Fig. 3.—Glomerulus of rat four months after period of choline deficiency. Thickening of the capillary walls is somewhat more pronounced than in Figure 3. PAS stain; reduced 5% from mag. × 875.

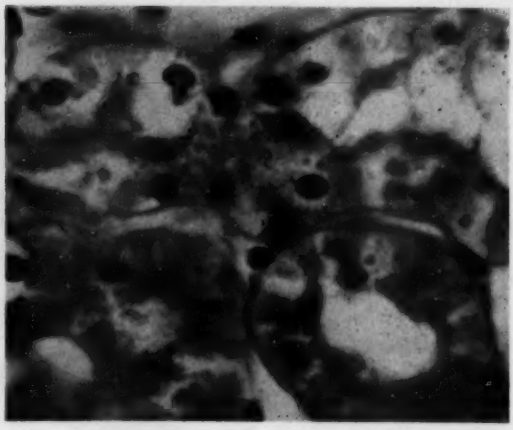


Fig. 4.—PAS-positive droplets in the cytoplasm of cells of the proximal convoluted tubules; six months after choline deficiency; × 875.

juxtaglomerular apparatus (Fig. 5). By this method, better visualization of the granules was obtained than with the methyl violet stain recommended by Wilson [5] and used by Hartroft and Hartroft.[6] No significant increase or decrease of these granules was found in the earlier stages after the period of choline deficiency, prior to the onset of hypertension.

The rise of blood pressure to hypertensive levels which follows choline deficiency is a gradual one,[3] and, as noted in the Table, the average blood pressure is only slightly elevated at two months, while many animals still have a blood pressure in the normal range. However, renal damage of a considerable degree in the form of glomerular capillary wall thickening is already evident at this time. At 9 months of age, most animals are definitely in the hypertensive range (average B. P., 150 mm. Hg). Prior to this time prominent and progressively increasing glomerular fibrosis is observed.

Renal Changes in Chronic Hypertensive Animals.—The earlier lesions of the glomeruli (Fig. 2), prior to the onset of

Fig. 5.—Normal degree of PAS-positive granules in the juxtaglomerular cells of the afferent arteriole; six months after choline deficiency; × 875.

Fig. 6.—Glomerulus nine months after choline deficiency. Marked thickening of the capillary walls, pericapillary deposits of PAS-positive material, and increased prominence of the basement membrane can be seen; × 350.

Figure 5

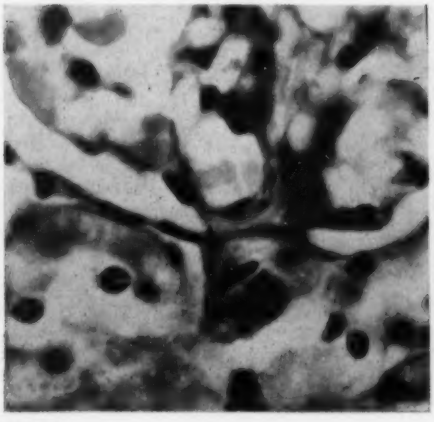
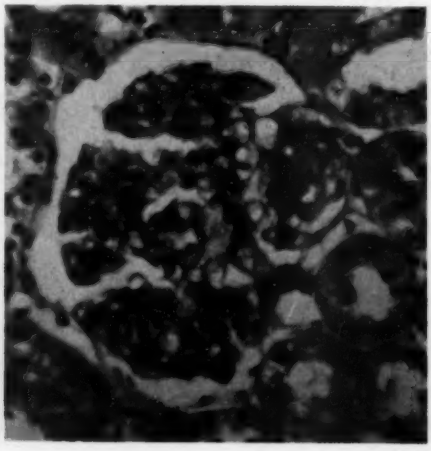


Figure 6



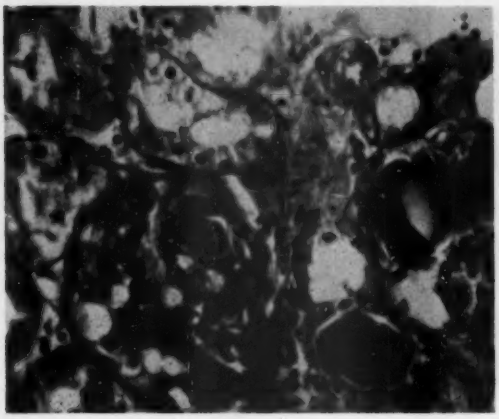


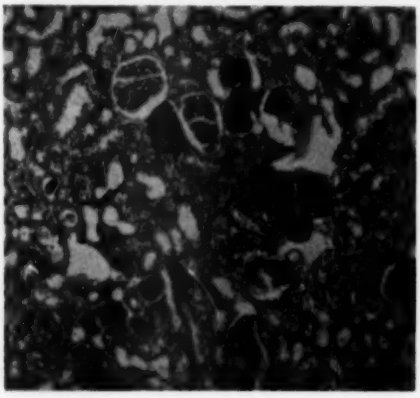
Fig. 7.—Marked fibrosis in glomerulus, with reduction of capillary space; one year after choline deficiency. B. P. 170 mm. Hg. Dilatation of tubules, atrophy of tubular epithelium, and tubular casts are also present. PAS stain; × 350.

definite hypertension, are qualitatively similar but of a less marked degree than those seen in animals with marked elevations in blood pressure. In chronic hypertensive rats, a year or more of age, there was a marked increase in PAS-positive material in the glomerular capillary walls (Figs. 6 and 7). This material, which stained blue with aniline blue and pale red with Van Gieson's stain, was located between the capillaries, was very uniformly deposited throughout all portions of the glomerulus and was associated with a prominent, thickened basement membrane. However, pericapillary fibrosis was more marked around the hilus of the glomerulus. The capillary lumina in the glomeruli were decreased in size and patency, and usually only a few red cells were present in the glomeruli. Many glomeruli of the chronic hypertensive animals contained adhesions between the glomerular tufts and the parietal layer of Bowman's membrane. The cellularity of the glomeruli varied in different animals and in different glomeruli within the same kidney; some were more cellular than normal, while others showed sparsely cellular glomeruli associated with denser collections of connective tissue matrix.

In addition to the changes in the glomeruli, other renal lesions, which were very marked in some instances, were noted in chronic hypertensive rats. The more advanced lesions showed extensive focal tubular dilatation, casts, interstitial fibrosis, and atrophic glomeruli (Fig. 8) similar to the "chronic nephrosis" described by Griffith and Wade.[1] In many animals, however, with hypertension of an equal degree, the tubular changes were much less marked or virtually absent. It was not unusual to encounter grossly normal kidneys, with histologically intact tubules and blood vessels, in rats with a systolic blood pressure up to 170 mm. Hg. In all of these, however, the glomerular fibrosis was unmistakably present.

Arteriolar and arterial changes, similar to those observed by Halpert and Grollman [7] and Muirhead et al.[8] in animals rendered

Fig. 8.—Cortex of kidney of rat one year after choline deficiency. B. P. 170 mm. Hg. Tubular dilatation and casts are prominent. PAS stain; × 80.



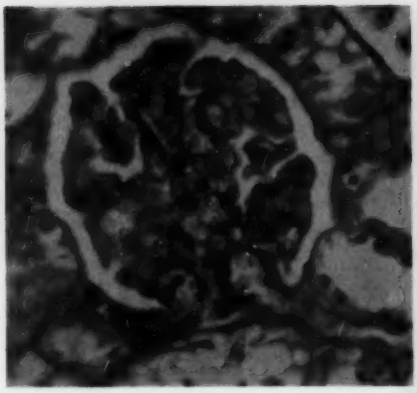


Figure 9

Fig. 9.—Glomerulus of hypertensive rat one year after choline deficiency. The afferent arteriole at the hilus of the glomerulus is dilated, and the wall is thickened and hyalinized. PAS stain; × 350.

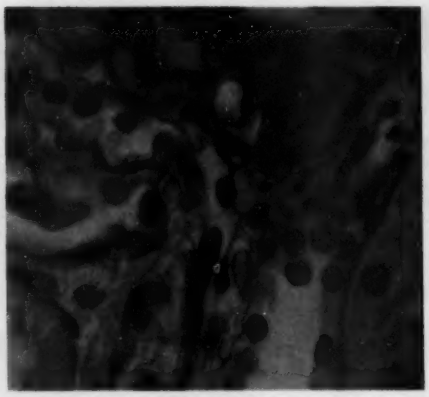


Figure 10

Fig. 10.—Vascular stalk and macula densa of glomerulus in hypertensive rat, one year after period of choline deficiency. The juxtaglomerular cells of the afferent arteriole reveal a pale, vacuolated, and almost completely degranulated cytoplasm. PAS stain; × 875.

hypertensive by other procedures and as described by Hartroft and Best [2] after choline deficiency, were also present (Fig. 9). The arteriolar changes were most prominent. The afferent arterioles were dilated, with hyalinized walls. Usually this dilatation was most marked at the site of entry into the glomerulus, and it extended into the precapillary segment within the glomerulus. In some, a fibrinoid appearance was found in the arteriolar wall. Other arterioles contained deposits of hyaline material, with nuclear pyknosis and fragmentation in the intimal zone. The small arteries of the kidneys showed slight fibrous thickening of the intima but no lipid deposits. The juxtaglomerular cells in most animals with well-established hypertension showed a marked reduction of PAS-positive granules (Fig. 10).

In a few animals which had received a low-choline diet as weanlings but which manifested only slight hypertension marked glomerular changes were nevertheless present. It was not possible to differentiate these kidneys histologically from those of animals with marked hypertension.

Alterations related to the prior choline deficiency or to the hypertension were not striking in organs other than the kidney except for left ventricular cardiac hypertrophy, arteriolar sclerosis, and slight arteriosclerosis in many of the chronic hypertensive rats.

Comment

The mechanism through which choline deficiency results in hypertension in rats has not been definitely established. Hartroft and Best [2] considered the reduction of glomerular capillary bed, induced by choline deficiency, to be the determining factor in the degree of hypertension. They emphasized the tubular destruction and marked dilatation of nephrons and ascribed the thickening of the glomerular basement membrane to the hypertension.

The present study indicates that almost immediately after or during choline deficiency glomerular injury occurs, resulting in swelling and proliferation of the glomerular epithelial cells, followed by the elaboration of a thickened basement membrane and collagenous matrix. At this stage, evidence of persistent tubular injury is not found. Griffith and Wade [1] and Christensen [9] observed evidence of glomerular injury caused by choline deficiency in rats, and it has been suggested that glomerular changes in the

human with portal cirrhosis [10] might be due to choline-deficiency or other deficiency states. In studies upon the acute renal injury produced in weanling rats by choline deficiency, Moore [11] showed that glomerular injury and even necrosis occurred. This was considered to be due to ischemia. Thus, the early glomerular lesions in rats that ultimately developed hypertension after choline deficiency seem to be a direct effect of choline deficiency itself; the late, severe glomerular changes of such animals may be the chronic expression of this earlier injury.

Glomerular lesions of the type described here are usually explained as the result of hypertension.[2] This explanation is difficult to accept in the case of choline deficiency because of the early occurrence of the renal lesions and their presence in animals in which hypertension has not developed. It seems more probable that the glomerular damage is a direct consequence either of the metabolic disturbance induced by choline deficiency or of some disturbance in blood flow to the kidney, resulting in ischemia. Such ischemia would not necessarily be due to, or immediately related to, hypertension. Fulton and Lee [12] have shown that in rats subjected to acute choline deficiency arteriolar constriction in mesenteric vessels is demonstrable by direct observation. An increased sensitivity of arteries and arterioles to epinephrine was also shown to be present in these animals. A similar effect upon renal arteries and arterioles, in choline deficiency, might be postulated to occur.

Glomerular lesions of a nature somewhat similar to that observed in choline-deficient weanling rats are known to be associated with the occurrence of hypertension in the human. Such lesions are observed in glomerulonephritis, essential hypertension, chronic pyelonephritis, eclampsia, amyloidosis, and disseminated lupus erythematosus.

Since some hypertensive rats were encountered in which no significant tubular changes were found and only glomerular fibrosis was present, it would appear that tubular injury is secondary to glomerular damage in these animals. In several instances proximal convoluted tubules were found to be the seat of extensive hyaline droplet formation. This would suggest that marked loss of protein was occurring in the glomerular filtrate, with partial tubular reabsorption of the protein. Such proteinuria was demonstrable in these animals. Much of the protein continues into the distal end of the tubules, where, as a result of the reabsorption of water, numerous protein casts form in the more severely damaged kidneys. Dilatation of the tubules appears to result from this obstruction, with subsequent atrophy of the epithelium of the dilated tubules. The stage of "chronic nephrosis" of Griffith and Wade [1] is thus attributable to the glomerular injury which induces proteinuria and tubular obstruction by casts.

Our findings indicate that the reduction in the number of glomeruli and the reduced glomerular capillary bed, as described by Hartroft and Best,[2] actually represent a late stage and are associated with the parenchymal destruction that occurs with the obstructive tubular process. Reduced glomerular capillary flow may occur later, however, as a result of the pericapillary fibrosis and cellular proliferation in the glomeruli.

Observations made here upon the special cells of the juxtaglomerular arterioles do not allow any definite conclusion as to their function and significance. It appears that injury of these cells by choline deficiency does not occur to any significant degree, since the cells appeared to be intact and normally granular immediately after and for several months after the period of dietary deficiency. That the juxtaglomerular cells are related in some manner, however, to the state of hypertension is suggested by the degranulation found in animals with well-established hypertension.

Conclusion and Summary

In rats fed a choline-deficient diet as weanlings, chronic hypertension is regularly produced. This may occur without the

severe hemorrhagic lesion induced by prolonged choline deficiency. Within two weeks after the low-choline diet, the glomeruli exhibit a progressive reduction of capillary bed, with thickening of the basement membrane and proliferation of the glomerular epithelial cells. Within a few weeks, a matrix of fibrous tissue begins to form. This glomerular lesion precedes the onset of hypertension. Later, the glomeruli become extensively involved with fibrous replacement of much of the glomerular capillary system. Hypertension is usually present at this stage. Later, when chronic hypertension has become established, many of the renal tubules are obstructed by protein casts and dilated and the tubular epithelium undergoes atrophy. At this stage, a picture very similar to the vascular, tubular, and interstitial tissue changes of severe arteriolar nephrosclerosis of the human is observed. The glomerular fibrosis, however, is severer, is more constantly present, and is more diffuse than that seen in the glomeruli of the human with arteriolar nephrosclerosis.

Although this study does not localize with certainty the part of the nephron, injury of which is responsible for the hypertension which follows choline deficiency in rats, the glomerular injury is the earliest demonstrable persistent lesion. It appears on the basis of our observations that the glomerular lesion is the result of choline deficiency, rather than secondary to hypertension. Interference with the blood supply through the glomerulus, or some other disturbance of an as yet unidentified portion of the kidney secondarily affected by the glomerular injury, would, according to this view, be responsible for the development of hypertension. The disappearance of granules from the juxtaglomerular apparatus may perhaps reflect some aspect of the renal dysfunction responsible for the development of hypertension.

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A Teratoma Arising in the Stomach in a Young Infant

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The rarity of gastric teratoma warrants the reporting of this case. We have found previous references to only two such cases. Selman [1] reported a gastric teratoma which was successfully removed from a boy 3 months old, and Large and others [2] recorded a similar tumor in a 7-month-old infant, successfully treated by a subtotal gastrectomy.

Report of a Case

A 6-week-old boy was admitted to the Childrens' Hospital, Colombo, Ceylon, on Oct. 7, 1957, with a history of vomiting after feeds. He was the youngest child (born at term) in a family of six. The third pregnancy was a stillbirth at full term, but the other children are alive and well. The delivery was normal, and birth weight was 7 lb. 4 oz. (3,290 gm.). The infant, who was breast fed, began to vomit his feeds on the 10th day after birth. The vomit was projectile in character. After a spontaneous cessation of vomiting for a period of four weeks he steadily began to lose weight—his weight now being 5 lb. 4 oz. (2,380 gm.). At the time of admission to the hospital vomiting had recommenced. There was no hematemesis.

Examination

On examination the child was very feeble and there was evidence of marked dehydration. The heart appeared to be displaced to the right side. The left side of the chest was hyper-resonant. Although the abdomen was distended, there was no visible peristalsis. Besides the liver, which was palpable 2 fingerbreadths below the costal margin, there were three separate nodular lumps palpable in the epigastrium. A characteristic feature was that these masses appeared and disappeared from the abdomen from time to time.

A skiagram of the chest confirmed the displacement of the heart to the right side. There was, however, no evidence of a pneumothorax, and the diaphragm could not be defined.

Although the child did not vomit during his three-week-stay in the hospital, he never took more than 2 fluid ounces of milk at any one feeding. In spite of intravenous therapy and blood transfusions his feebleness increased, and he died after 21 days in the hospital.

Autopsy

Autopsy findings were confined to the thoracic and abdominal cavities. The heart was slightly displaced to the right, and a portion of the stomach had herniated through the esophageal hiatus into the thoracic cavity and become adherent to the left lung, the base of which was collapsed.

When the stomach was opened a large tumor was found to occupy more than one-half of it (Fig. 1). It extended from the fundus and ap-

Fig. 1.—Stomach opened to show large cystic tumor. Note the adherence of stomach to left lung.

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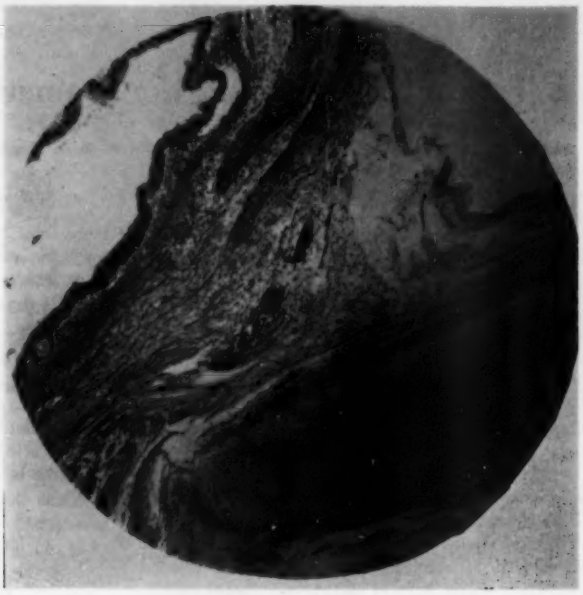

Fig. 2.—Section of the tumor, showing epithelial-lined cystic spaces as well as cartilage.

peared to arise from the posterior wall of the stomach. The serosa was not infiltrated. The sectioned surface of the tumor showed several cystic spaces, some of which were filled with bluish black semifluid contents. The intervening areas were hard and cartilaginous.

Microscopic Appearances

The tumor, which consists of a conglomeration of tissues, is lined on its superficial aspect by cellular granulation tissue in which gastric mucosal remnants are visible. The tumor is composed of several cystic spaces lined by columnar ciliated, cuboidal, and squamous epithelium. Occasional hair follicles, as well as cysts lined by mucous-secreting epithelium, are made out. Smooth muscle and masses of cartilage are conspicuously seen (Fig. 2).

Comment

The pathological evidence leaves no doubt that this is a teratoma arising from the stomach wall and that, as in the two cases previously described in the literature, all three germinal layers participated in the neoplastic process. A peculiar feature, however, in this case was that almost half of the stomach, bearing the tumor, had herniated through the esophageal hiatus into the thoracic cavity, giving rise to a diaphragmatic hernia. This was the probable cause of vomiting and the presenting symptom and also accounted for the hyper-resonance on the left side of the chest as well as for the appearance and disappearance of the abdominal tumor. In the case recorded by Large and others [2] hematemesis and melena were the presenting symptoms, and in Selman's [1] case the tumor was discovered at a routine examination. The age at which the condition was recognized in the present case (viz., 6 weeks) is the lowest recorded. Both previously recorded cases were treated successfully, one by removal of the tumor and the other by subtotal gastrectomy. Unfortunately in this case the young age of the infant and his feeble state did not warrant any operative procedures.

Summary

This is the third case recorded of a gastric tridermal teratoma. An unusual feature of this case was that the tumor-bearing portion of the stomach had herniated into the thoracic cavity.

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The Relationship Between Eye and Kidney Pathology in the Diabetic Rat

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The literature pertaining to microangiopathy in diabetes has been reviewed recently by Ashton.[1] Although several investigators have shown that vascular lesions may occur in various parts of the body in uncontrolled or poorly controlled human diabetics, the chief disability is usually in the retina and kidney. Friedenwald[2] and Ashton[3] first pointed out the close association between retinal microaneurysms and Kimmelstiel-Wilson (K-W) nephropathy.

Since these lesions are closely related to one another in the diabetic human being, several workers have attempted to find them in diabetic animals. Although Lewis et al.[4] found minute retinal hemorrhages in diabetic rabbits, this observation was not confirmed by other workers.[5,6] Glomerular changes which may resemble the K-W lesion have been described for the diabetic rat by Foglia et al.,[7] Mann and Goddard,[8] Mann et al.,[9] and Janes.[10] However, Curtis et al.[11] were not able to find glomerular alterations in this animal. Moreover, the kidney of the diabetic rabbit apparently does not show these lesions.[12]

The desirability of being able to produce glomerular and retinal lesions in a diabetic animal is very apparent. This would enable one to set up experiments in an attempt to modify these pathological changes and perhaps give some clue as to their etiology. Although retinal microaneurysms are not usually seen in the diabetic rat, certain vascular alterations of the eye have been described.[13] The present study was undertaken in order to compare the pathogenesis of vascular lesions of the eye and kidney of the diabetic rat.

Material and Methods

Adult rats of the Long-Evans strain, which had been inbred for 30 years, were used in this study. After a 48-hour fast the animals were given subcutaneous injections of 115-125 mg. of alloxan per kilogram of body weight. After one week, blood sugar levels, urine volumes, food intake, and body weights were determined. Over 200 animals having blood sugar levels of at least 250 mg. % were used in the studies. The severity of diabetes was checked at least once a month by determining blood sugar levels and/or presence of glycosuria. Animals which did not maintain high blood sugar levels or a 4+ urine sugar were discarded. The kidneys and eyes of the diabetic animals were studied for periods of one week to 18 months. The rats were fed Rockland Rat Ration or a synthetic diet[14] complete in all essential food substances.

The eyes were examined periodically with an ophthalmoscope or slit lamp to observe the development of cataracts and vascular alterations in the anterior segment. After two to three months it was impossible to observe the retinal vessels because of lens opacities. Retinal abnormalities thereafter were studied in whole mounts or sectioned material into which India ink or neoprene latex had been injected. These opaque materials were injected into the thoracic aorta, India ink with 4 lb. of pressure and latex with 10 lb. of pressure. Within a few seconds after the injection was started the materials appeared in the eye. The heads were submerged immediately in a 15% formol solution for 24 hours, and 2 hours after the injection a small amount of 10% formol was injected into the region of the posterior chamber of the eye. The eyes were enucleated and opened coronally near the ora serrata. The anterior segments of the eyes were bleached with 3% potassium permanganate and 2% oxalic acid. Ordinarily the

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cornea, iris, and retina were mounted flat in glycerin jelly. In certain instances the anterior segment of the eye was fixed in Bouin's fluid, embedded in paraffin, and sectioned.

The kidneys were removed usually before the eye vessels received injections, and parts were fixed in 10% formol and Bouin's fluid. In certain cases unstained sections of kidneys were examined with the color-translating ultraviolet microscope.[18] Sections of kidneys were stained with Mallory's trichrome, Masson's trichrome, allochrome, and periodic acid-methenamine techniques and examined under the light microscope. Some sections were stained for lipids with Sudan black or osmic acid.

Results

Angiopathy of the Eye.—A. Retina: Retinopathy per se was rarely encountered in the diabetic rat. Ophthalmoscopic examination of over 200 diabetic animals revealed a striate hemorrhage in only 1 animal, which had been diabetic for eight weeks.

In specimens which had received injections one eye from each of two animals had aberrant retinal vessels. These vessels extended into the vitreous (retinitis proliferans?) and probably had no connection with the embryonic hyaloid circulation. Perhaps they were an embryonic defect or were caused by the diabetes per se.

Particular attention was given to the retinal vessels in the specimens given injections. Typical microaneurysms, as have been commonly found in human diabetics, were not seen. In fact, the retinal capillaries did not show any evidence of fragility. Ordinarily they did not even burst during the injection of the opaque medium.

A difference was noted, however, in how well the retinal vessels became filled with neoprene latex in normal and severely diabetic rats. In normal animals all of the vessels were generally filled with latex (Fig. 1). However, animals having been severely diabetic for at least four months often showed filling of only the retinal arteries and arterioles (Fig. 2). Various degrees of filling were observed in animals with diabetes of shorter duration or when the diabetes was of a milder type. Apparently the failure of the venous side of the retinal tree to fill normally was either because of increased resistance in the capillary bed or because of a reduction in the pressure or amount of latex reaching the retina. The retinal circulation, however, was still intact

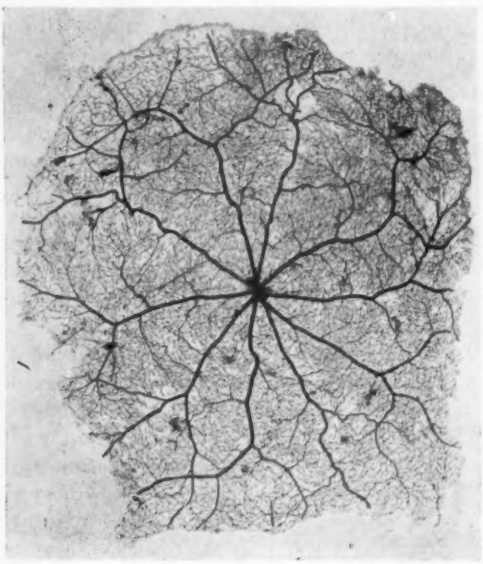


Fig. 1.—Whole mount of retina from normal rat. All blood vessels are filled with latex.

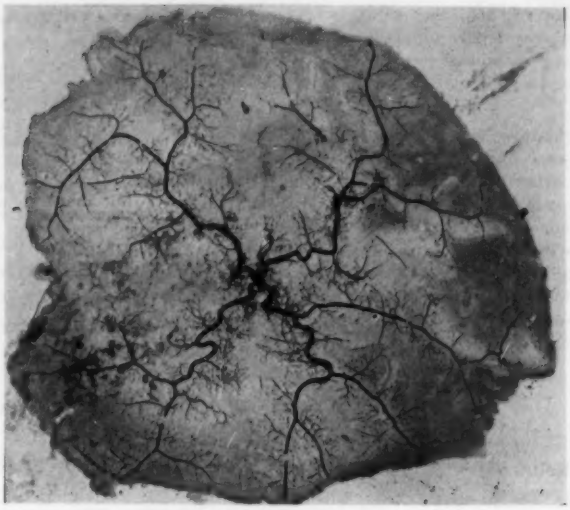


Fig. 2.—Whole mount of retina from rat diabetic for four and one-half months. Only retinal arteries and arterioles filled with latex. There are usually five to seven retinal arteries, but this animal had only four.

in the living animal because the vessels usually filled well with India ink.

B. Anterior Segment of the Eye: Hemorrhage into the anterior chamber of the diabetic rat's eye was described earlier.[13] In the present work more animals with hemorrhage have been observed, and additional details of the pathological changes have been made. A total of 42 rats, having had severe diabetes for at least four months, showed mild-to-massive hemorrhage into the anterior chamber (Fig. 3). The blood rarely passed into the posterior chamber or vitreous body. Sometimes the bleeding was intermittent and blood was absorbed before additional hemorrhages occurred. At other times, the bleeding was continuous, producing a bulging of the eye. In fact, in two of the latter rats the cornea became eroded and the contents of the eye were expelled. Hemorrhages were never observed in the anterior chamber of diabetic rats until they had a mature cataract.

Fig. 3.—Rat with hemorrhage in anterior chamber of left eye. Note blood, which appears whitish, in lower half of this eye.

The origin of the hemorrhage was not clear. Eyes with hemorrhage were dissected and examined carefully, and the anterior segments were sectioned and stained. In no instance was it possible to discover which vessel or vessels were bleeding. Whole mounts of eyes from animals that had been severely diabetic for the length of time usually required for the hemorrhage to occur were studied in detail after an opaque medium was injected into the vessels. The first thing that became obvious was that the major arterial circle and some of the ciliary vessels were dilated (Fig. 4). The normal vascular pattern was described earlier.[16] An aneurysmal-like dilation had been noted in one iris,[13] close to the major arterial circle. Presumably such a dilation represented a weakness in the wall of this part of the vessel. Furthermore, when latex was injected under 10 lb. of pressure there were usually more "blowouts" (latex rupturing from the vessels) in the diabetics than in

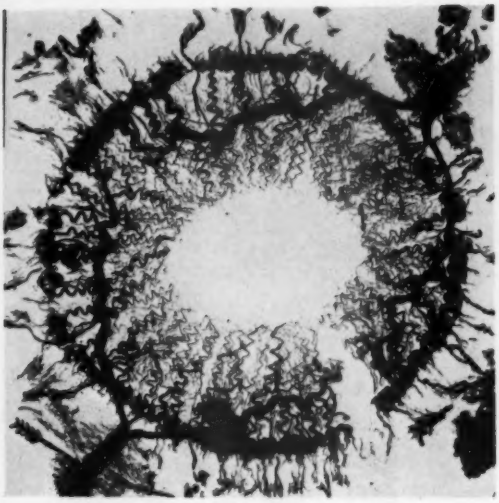


Fig. 4.—Whole mount of iris and ciliary body from rat diabetic for eight months. The long posterior ciliary arteries (arrow) and major arterial circle are distended. There is a "blowout" in either the major arterial circle or the ciliary artery in the lower side of photomicrograph.

the normal rats. These "blowouts" occurred ordinarily along the major arterial circle and/or in the pupillary part of the iris vessels. One such "blowout" is seen in Figure 4.

Marked pathologic changes were present in the anterior segment of the eye soon after the hemorrhage occurred. Within a week there was always an ingrowth of vessels from the limbal arcade into the cornea (Fig. 5). If blood stayed in the anterior chamber for a greater length of time the entire cornea became vascularized (Fig. 6). The endothelial lining of the anterior chamber became eroded in some specimens, and fine connective tissue membranes, similar to those found after the organization of a clot, in many instances nearly filled the anterior chamber.

Some of this vascularized connective tissue was attached to the iris and formed a secondary pupillary membrane (Figs. 5 and 6). Synechiae were commonly observed. These pathological changes probably resulted from the reaction of the tissues to the blood in the anterior chamber, and, in addition, the apparent increase in intraocular pressure may have been a contributing factor.

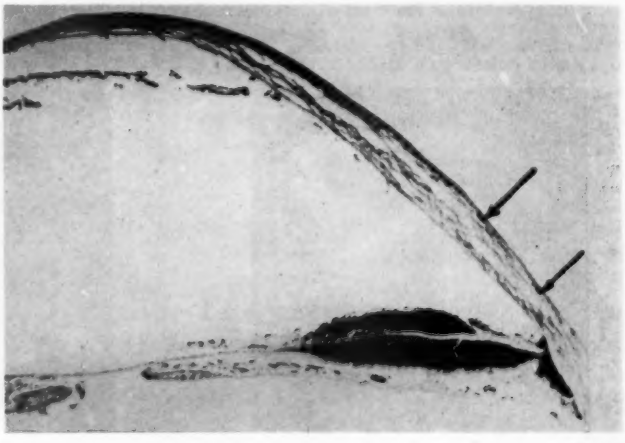


Fig. 5.—Section of anterior segment of eye. Hemorrhage present for seven days. Note clot on anterior surface of iris, some blood cells near cornea and secondary pupillary membrane. Small blood vessels, into which India ink has been injected, may be seen in cornea (arrows).

Fig. 6.—Section of anterior segment of eye. Hemorrhage present for seven weeks. The cornea is thickened and vascularized. Two clots are organized, and the iris angle is filled with connective tissue. A secondary pupillary membrane is present.

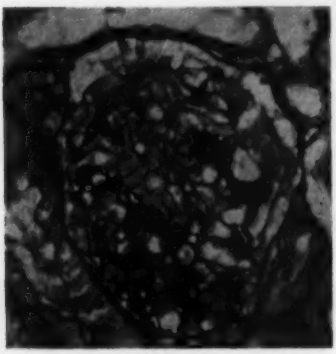


Fig. 7.—Glomerulus from rat diabetic for four and one-half months. Adhesions of glomerulus with parietal layer of Bowman's capsule in upper part of section. Some capillaries are dilated, and walls show early thickening. Allochrome stain; × 284.

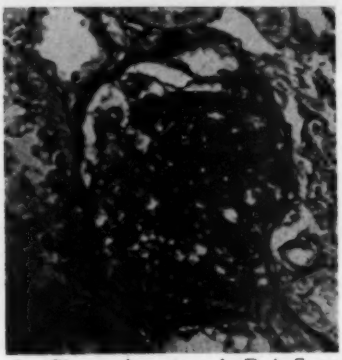


Fig. 9.—Glomerulus, strongly P. A. S.-positive, from rat diabetic for five months. Thickening of basement membranes of both parietal and visceral layers of Bowman's capsule. Some diffuse thickening of capillary walls. Allochrome stain; × 284.

Fig. 8.—Glomerulus from rat diabetic for five months. Shows early stage of diabetic glomerulosclerosis with diffuse thickening of capillary walls. Allochrome stain; × 284.

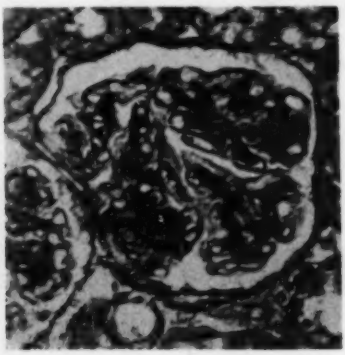
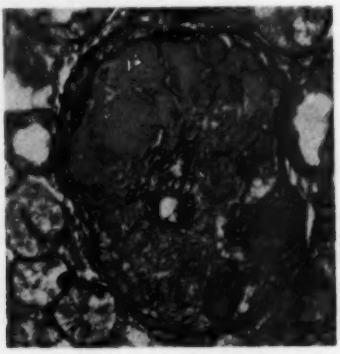


Fig. 10.—Glomerulus from rat diabetic for 18 months. Nodular type of lesion, with central area nearly completely sclerotic. Allochrome stain; × 284.



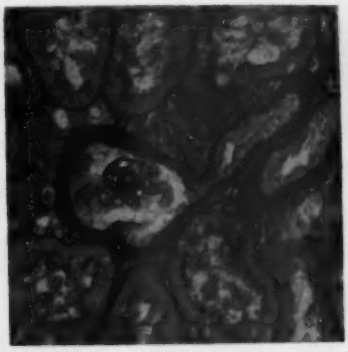


Fig. 11.—Glomerulus and proximal convoluted tubule from rat diabetic for five months. Shows atrophy of glomerulus and marked thickening of the basement membrane of Bowman's capsule and of the proximal convoluted tubule. Allochrome stain; × 284.

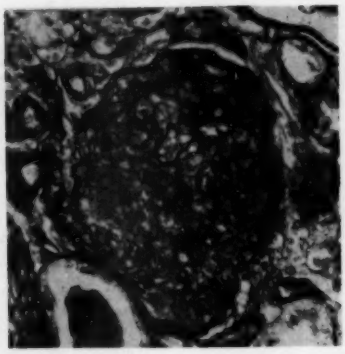


Fig. 12.—Glomerulus from rat diabetic for 18 months. There is a marked reduction in number of capillaries. Glomerulus is sclerotic and shows little shrinkage. Allochrome stain; × 284.

Sequence of Eye and Kidney Changes.—Aside from the metabolic changes of diabetes, the first clinical sign exhibited by rats was the appearance of cataracts. By the time the cataracts were nearly mature (three months or longer), definite changes were seen in the kidney. The kidney alterations were largely confined to the renal corpuscles.

One of the first signs of glomerular alterations was the thickening of capillary walls and dilation of some capillaries (Fig. 7). Occasionally what appeared to be an amorphous exudate was seen, but this probably represented a focal thickening of the capillary wall (Fig. 8). A few renal corpuscles had marked thickening of the parietal basement membrane of Bowman's capsule (Figs. 9 and 11), and the basement membrane of the proximal convoluted tubules which were associated with these corpuscles was also thickened (Fig. 11). A small number of glomeruli were partially or completely atrophied (Fig. 11).

After hemorrhages had occurred in the anterior chamber of the eye, which meant that the diabetes had caused vascular damage, the pathological alterations in the kidney were much severer. In some animals the histological picture was similar to that found in human diabetic glomerulosclerosis, except that the lesions were not so circumscribed (Fig. 10). Actually there were very few normal glomeruli, and the pathological ones exhibited much variation, from mild to marked sclerosis. Mildly sclerotic glomeruli had thicker capillary walls than normal (Figs. 8 and 9), and with Mallory's stain the stroma appeared quite granular. Silver and periodic acid-Schiff (P. A. S.) stains, however, showed there had been an increase in reticular fibers (Fig. 9). Many of the glomeruli had dilated capillaries (Figs. 8 and 9), and some capillary walls showed evidence of a recent focal thickening, which was P. A. S.-positive.

The glomeruli having a marked sclerosis showed very little shrinkage, i. e., Bowman's space was small. Also, very few glomerular capillaries were visible in such specimens (Figs. 10 and 12). Some glomeruli showed P. A. S.-positive nodular lesions toward the parietal basement membrane (Fig. 10). Others showed just a granular and fibrillar type of lesion with few capillaries. Of course, renal corpuscles were present in which the glomeruli were partially or completely atrophied (Fig. 11).

The kidney tubules were pathologically altered. Some had undergone partial degeneration, i. e., the epithelial cells were sloughed. Others were markedly dilated or atrophied and replaced with connective tissue. In kidneys with a severe pathology,

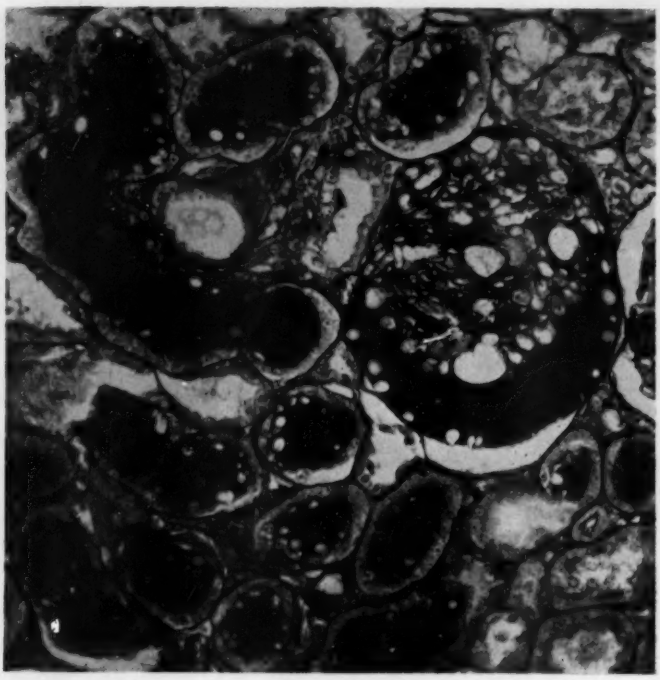


Fig. 13. — Glomerulus and associated proximal convoluted tubule from rat diabetic for 11½ months. Glomerulus shows dilated capillaries and focal thickening of capillary wall. Material staining black is an exudate of proteinoid nature. Allochrome stain; reduced 5% from mag. × 284.

cell casts, an exudate, or a granular coagulum were found often in the collecting tubules. (Fig. 13 shows exudate.) The epithelial cells of many convoluted tubules and the thin segments of Henle's loop contained glycogen.

The blood vessels of the diabetic rat seem to be unusually resistant to arteriosclerotic changes. Nevertheless, in examining the kidney vessels it was found that the walls of some arteries and arterioles, including the afferent and efferent glomerular arteries, showed a moderate-to-marked thickening, and a few arteries showed some hyalinization in the tunica media. Hemorrhage per se was not encountered in the kidney unless a localized infection was present.

Certain other observations are important in this study. The blood sugar levels ranged from 250-650 mg. % for periods of months. Some rats excreted in their urine as much as 15 gm. of glucose per day. Furthermore, many rats showed a consistent ketonuria, but when this became too severe the animals would succumb to the disease. In addition, rats having severe diabetes for many months often showed albuminuria and hematuria. Again, if these conditions lasted too long or became too severe the animals died. Actually the K-W-like lesion described was present in the animals exhibiting an albuminuria and hematuria. All in all, however, the animals withstood the diabetes very well, considering they were not given insulin.

Comment

The pathological alterations which lead to retinopathy and K-W nephropathy in the human diabetic exhibit a somewhat different pattern in the diabetic rat. Although the kidneys may show lesions similar to those in the human being, the retina of the rat seems to be virtually unaffected by the disease. On the other hand, diabetic rabbits may show retinal hemorrhages, according to Siliato,[17] but it is doubtful if the hemorrhages noted by this worker are similar to those of the human being.[1]

Also, Lewis et al.[4] described minute retinal hemorrhages in diabetic rabbits, but

this finding has not been verified by other investigators.[5,6]

Intraocular hemorrhages, some perhaps similar to the ones described in this study, have been observed in weanling rats which have been given a choline-deficient diet. Important papers in this field are by Griffith and Wade[18] and Bellows and Chinn.[19] In the present study, however, it is questionable whether choline deficiency had anything to do with the hemorrhage. All of the rats used were adults, and some were fed a balanced rat ration. Others were given a complete synthetic diet which was supplemented with choline chloride. Hyphemas occurred at about the same rate in these two groups of animals.

There is a possibility that the iris vessels may be damaged because of hypoxia in the diabetic animals. It has been pointed out in this study that there is some congestion in the major arterial circle of severely diabetic rats. If this means that there is a curtailment in blood supply to the iris and ciliary body, it could mean also that there is a reduction in the amount of oxygen reaching these vessels supplying these structures. If this is the case there is a good chance that these vessels would rupture in weakened areas.

Lesions in kidney glomeruli, thought to resemble diabetic glomerulosclerosis in man, have been observed in diabetic rats. Mann and Goddard,[8] Foglia et al.,[7] and Mann et al.[9] have described such lesions, but Curtis et al.[11] were not able to find glomerular lesions in animals having alloxan diabetes for long periods of time. The lesions described by the above workers do not seem as severe as the ones reported in the present study. The difference may be in the strain of rats used and/or the duration of severe diabetes.

Glomerular nephropathy in the rat appears to start in the capillary wall. This becomes thickened, and focal and diffuse lesions appear in relation to the capillaries. Dilated capillaries are found in some glomeruli. Later, about the time when hemorrhages appear in the anterior chamber of the eye, large amorphous hyalin masses appear in the glomeruli. Eventually many of the affected glomeruli may atrophy or become almost completely sclerotic.

Hartroft[20] has suggested that the glomerular lesion in choline-deficient rats and in patients with diabetic glomerulosclerosis may be caused by fat emboli. Special attention was given in the present study to the distribution of fat in the diabetic kidney. Both osmic acid and Sudan black preparations were made of kidneys during the progress of glomerular alterations. In no instance were fat emboli noted in any glomerular vessels. The only lipid found in glomeruli was in connective tissue macrophages.

Thus, a definite chronological association has been found in the diabetic rat between the changes in the eye and kidney. However, these changes are not strictly comparable to those found in the human diabetic. It appears that these lesions evolve or result from vascular damage. In the kidney this damage allows for abnormalities in the glomerulus and in the eye the rupturing of a vessel or vessels into the anterior chamber. The cause of this vascular damage is not clear.

Summary

Diabetic rats of the Long-Evans strain show a relationship between the pathological changes of the eye and kidney. By the time cataracts are mature definite kidney glomerular lesions are present. Later, certain vessels may rupture into the anterior chamber of the eye, causing marked alterations in this organ. If the kidneys are examined in such animals, glomerular alterations are very severe. In fact, many renal corpuscles take on the appearance of Kimmelstiel-Wilson nephropathy. The lesions in both the eye and kidney can be attributed to abnormalities of blood vessels, the cause of which is not clearly understood.

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Choledochus Cyst in a Newborn

Report of a Case with Necropsy Findings

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The choledochus cyst represents a congenital, idiopathic, cystic dilatation of the common bile duct.[5] The first case was studied by Vater, cited by Tsardakas,[21] in 1723. In a recent review made by Horne [8] the total number of cases [1,4,13,16,18,19] was raised to 245, up to 1957.

We take advantage of an additional case herein reported to survey the subject once more, giving also a possible explanation about its pathogenetic mechanism.

Report of Case

A full-term boy, weighing 3,150 gm., was admitted to the hospital immediately after a difficult and complicated delivery. The family history was not contributory. Most of the boy's face and scalp was occupied by a fresh ecchymosis, while the upper third of the right humerus was the site of a complete transverse fracture. An extensive and marked abdominal distention was present owing to a large cyst the size of a newborn's head, as shown by palpation, percussion, and fluoroscopic examination. Upon paracentesis about 200 ml. of a serosanguinous fluid containing 0.7 gm. of protein per 100 ml. was withdrawn; culture preparations were negative, and numerous blood cells were found. Routine peripheral blood studies showed hemoglobin, 11 gm/100 ml.; erythrocytes and leukocytes, 4,200,000 and 5,500 per cubic millimeter, respectively, with a normal differential count; blood platelets, normal in appearance; serum total protein, 4.4 gm/100 ml., with the following electrophoretic pattern: albumin, 48.3%; α_1-globulin, 8.2%; α_2-globulin, 12%; β-globulin, 18%. A decrease of the albumin fraction was evident. The α_1-globulin was increased; urine protein, 1.0 gm/100 ml.; bleeding time, 3 minutes; clotting time, 8 minutes; Rumpel-Leede test, negative; clot retraction, normal.

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A tentative clinical diagnosis of ascites and congenital abdominal cyst of unknown etiology was made. The child was put on the critical list from the first day. The second day a slight jaundice was present; antibiotic and supporting medication was provided; he died the same day after a rapid downhill course characterized mainly by respiratory and circulatory distress.

Necropsy Findings

The body was that of a well-developed and not emaciated baby; moderate cyanosis of the mucous membranes and nail beds was present; the lower extremities were edematous. The fingers and toes showed no clubbing. No lesions of note were found in the dura, leptomeninges, or brain, which weighed 450 gm. A recent transverse complete fracture of the upper third of the right humerus and a small hemorrhage of the surrounding soft tissues were seen. No periostitis, osteolytic processes, or other pathologic lesions were found. The bone marrow was within normal limits. In each pleural cavity 4.5 ml. of hemorrhagic fluid was present. The external cut surfaces of the lungs were similar, covered by a smooth and glistering pleura, transmitting a light brown-yellow color, which was less pronounced at the anterior edges. Confluent, consolidated areas and a hemorrhage up to 1.2 cm. in diameter were demonstrated especially at the lower lobes. On sectioning, crepitance was present. The color was mottled light brown with a slight yellow cast. The bronchi contained a small amount of a yellow mucoid material. The hilar lymph nodes were normal. The thymus weighed less than 10 gm. but was of normal shape.

Microscopically, the pulmonary interstitial tissue showed scattered hemorrhages and leukocytic infiltrations consisting mainly of neutrophils and lymphocytes. A number of alveolar spaces were collapsed or distended. The thyroid was of normal size and shape. The heart, weighing 20 gm., showed a localized interventricular defect, 0.2 cm. in diameter, at the base of the septum, anterior to the pars membranacea. Patent ductus arteriosus, 0.2 cm. in diameter, was seen.

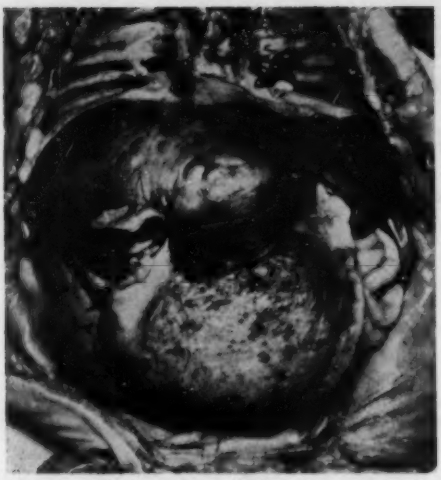

Fig. 1.—The picture is taken after a foreward flexion of the lumbar region in order to visualize the relationship of the liver to the cyst.

Microscopically, the myocardium showed a minimal focal increase of the interstitial tissue and a few scattered small hemorrhages.

The upper peritoneal area, in the region of the pancreas, behind the stomach and duodenum was occupied by a huge cyst, 20 cm. in diameter. (Figs. 1 and 2). Extensive peritoneocystic adhesions were found, especially posteriorly. The peritoneal cavity contained about 450 ml. of hemorrhagic fluid. The cyst wall averaged 0.15 cm. in thickness. It contained about 1,500 ml. of a bile- and blood-stained fluid, mixed with fresh blood clots. The cyst cavity communicated with the hepatic and cystic ducts through valve-bearing and easily probed orifices. The gallbladder was smaller than usual, namely 2.2 cm. by 1.0 cm. by 0.5 cm. Its lumen was partly divided into two irregular alveolar spaces by a membranous septum. The bile content was condensed, and a few small stones were also noted. The intraduodenal part of the common bile duct could not be probed, although some propagation of the probe was possible into Vater's ampulla. Microscopically, the cyst wall consisted of a dense, fibrous, vascular connective tissue. A few smooth muscle fibers were intermingled. Hemorrhages and a small amount of pancreatic tissue firmly attached to the posterior external aspect of the cyst were found (Fig. 3). The stomach and the duodenum, especially the latter, were markedly dilated and elogated. The duodenum, 3.4 cm. in its largest transverse diameter and 24 cm. in length, encircled the cyst. It was firmly attached to the undersurface of the cyst and approached the remaining small and large intestine to the lateral aspect of the cyst. Almost the whole intestinal tract was displaced to that side, maintaining otherwise its normal structure. The liver, weighing 85 gm., except for a slight dislocation and congestion demonstrated no other gross abnormalities. Microscopically, a certain cloudly swelling of the liver cells and scattered areas of myeloid metaplasia, mainly of the erythroblastic type, were seen. The spleen, weighing 10 gm., showed a moderate congestion and small hemorrhagic extravasations. The right and the left kidneys weighed 15 and 16 gm., respectively, while their fetal lobulation and normal size were preserved. Microscopically, extensive cloudy swelling of the epithelium of the renal tubules, mainly of the convoluted ones, and small scattered hemorrhages were found.

Fig. 2.—The cyst as shown when the liver is lifted.

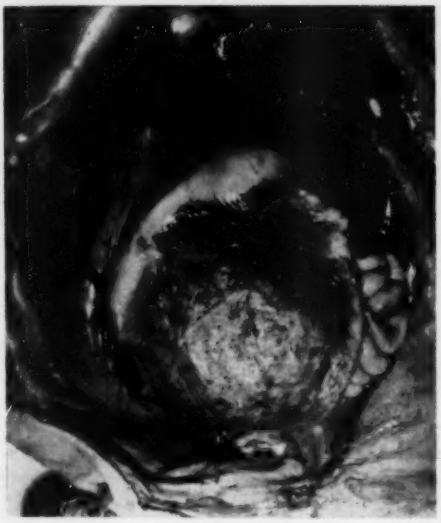

Fig. 3. — Photomicrograph of the cyst wall. *P*, pancreatic tissue; *V*, vessels; *I*, internal aspect; × 100.

The ureters, urinary bladder, urethra, and genitalia were not remarkable. The adrenals demonstrated an intense congestion, hemorrhages, cloudy swelling, and focal areas of necrosis. An extensive depletion of their lipoid content was also evident. Abdominal blood vessels and nerves exhibited a varying degree of compression and dislocation. Some small blood vessels, especially veins, were occluded by thrombotic material.

Final Diagnosis

Idiopathic cystic dilatation of common bile duct (choledochus cyst). Recent extensive, intracystic hemorrhages, apparently due to the difficult delivery. Extensively distorted anatomical relationships of the abdominal organs, resulting in elongation and distention of the duodenum. Hemohydroperitoneum. Edematous lower extremities and degenerative, nephrotic changes due to vascular compression. Localized defect of the interventricular septum. Patent ductus arteriosus. Focal pneumonia and small hemorrhages of the lungs. Fracture of the right humerus and cephalhematoma due to the difficult delivery.

Comment

The choledochus cyst is a rare anomaly of the common bile duct. Three cases were found among 50,000 admissions in the Children's Hospital of Pittsburgh.[8] Three-fourths of the published cases, strangely enough, were females. An average of 70% came to the doctor's office before the 25th year of age, and more than 60% have been encountered in children under 10 years old.[7]

The cystic dilatation involves mainly the upper portion of the common bile duct and very seldom the intraduodenal part or the cystic or hepatic ducts.[14] It is located retroperitoneally, on the anterior aspect of the pancreas and posterior to the duodenum, the latter exhibiting usually a pronounced dilatation. The gallbladder is often atrophic.[9] The size of the choledochus cyst varies from a few to 20 cm. From this point of view, the case reported herein was among the largest ones. In a case reported by Zinninger and Case, cited by Hutchins,[9] about 8 liters of fluid was removed from the cyst. The fluid content is usually both bile- and blood-stained. Stones are rarely admixed. The cyst wall, measuring from 0.2 to 0.6 cm. in thickness, consists of a dense fibrous connective tissue, including in rare instances a few smooth muscle fibers. The epithelial lining is rarely preserved.

An abdominal cystic tumor (77%), jaundice (70%), and pain (59%), located chiefly in the upper quarter of the abdomen, constitute the characteristic triad of clinical symptoms. The clinical diagnosis is not an easy task; in a series of 170 cases only

8.6% were diagnosed intravital, while the communication of the cyst with the bile ducts makes feasible the correct diagnosis on the autopsy table. The mortality rate is 100% in cases treated with paracentesis only, without operation or with operation not necessarily associated with anastomosis of the bile ducts to the duodenum. The mortality is 9% if the correct operation is performed.[6]

The anatomical relationships of the abdominal viscera, especially those of the gastrointestinal tract, are extensively altered. Ruptures and thromboses of blood vessels or rupture of the cyst itself have been occasionally mentioned. There are four such cases of spontaneously ruptured cysts with a subsequent choloperitoneum. Cirrhosis of the liver is often found.[3]

The cyst is usually congenital and idiopathic [11]; in rare cases, however, it may be acquired, secondary to some pathological process resulting from stenosis or complete occlusion of the common bile duct. The congenital weakening of the duct wall is likely to be involved in the pathogenesis of the cyst. Extensive reviews of the subject made by Shallow and co-workers,[15] in 1943, and by Böttger,[2] in 1951, do not clarify the etiology and pathogenesis of the cyst. Some similarity with the pathogenesis of idiopathic hydronephrosis, congenital megacolon, or megaloureter is considered probable by some writers. They also report that disturbances of Oddi's sphincter, causing subsequent dilatation of the common bile duct or further dilatation of a preexisting diverticulum, are seen by others. Irregular flexion of the intraduodenal part of the common bile duct or some other obstruction of the bile pathway is rarely cited. Yotyganagi expressed the view that irregular proliferation of the cells during the solid, embryonal stage of the common bile duct may account for its cystic dilatation.[1,15,17]

After a review of the embryology of the upper abdomen,[12] it seems logical to us that hepatic diverticulum, representing the primordium of the common bile duct, may itself be transformed to the cyst when dilated. The duodenal dilatation may possibly be a part of the whole anomaly or secondary to the intestinal obstruction mechanism due to choledochus cyst formation.

Summary

A rare case of choledochus cyst in a newborn infant is described. The child was born with a big abdomen, showing the clinical characters of ascites and a large abdominal cyst. A blood-stained fluid was withdrawn from the abdomen. The child died at the age of three days. At necropsy a large cyst of the choledochus duct connected with the hepatic and cystic ducts was found. It occupied the greater part of the upper abdomen and measured about 20 cm. in its maximum diameter. The cyst was full of a bile- and blood-stained fluid.

A short account of the incidence, clinical picture, and pathogenesis of the disease is also given.

Athanasios Mourdjinis, of Pathological Physiology, assisted in preparing this paper.

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Renal Angiomyolipomas

Report of Four Cases

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Of the various histopathologically interesting forms of hamartomas, renal angiomyolipomas represent a special type of such a growth in the kidneys. Although they are known to be relatively frequent in patients with tuberous sclerosis, reportedly in from 40%[9] to 80%[10] of such patients, only a few reports have been published pertaining to renal angiomyolipomas in patients without tuberous sclerosis. The few reports also reflect a certain confusion concerning the classification of these "growths." It is the purpose of this paper to present four cases of renal angiomyolipomas in patients without evidence of tuberous sclerosis and to discuss the intricate problem of classification of this type of hamartoma.

Hamartoma is defined as an abnormal development of mature tissue natural to the site or organ and represents a tumor-like nonneoplastic growth. The term is derived from the Greek word "hamartanein," i. e., to fail, to err, and was coined by Albrecht,[1] who described these "formations" as a "slightly distorted caricature of normal tissue" without certain features generally ascribed to true tumors.

The term *hamartoma* is, unfortunately, badly chosen because the suffix, *oma*, has been used generally to designate a true tumor, from which, by definition, the growth called hamartoma must be distinguished.

Renal hamartomas occur in two varieties: (1) the medullary fibroma and (2) the hamartoma of mixed mesenchymal origin. The latter occurs most commonly as a part of the tuberous sclerosis complex, and the tumors are usually small and multiple. However, as in the present cases, the mixed mesenchymal variant occasionally may occur in the absence of tuberous sclerosis and then is prone to occur as a single tumor.

Medullary Fibroma.—This lesion rarely attains clinical significance; it remains silent and asymptomatic and is most commonly detected at necropsy or incidentally in a specimen removed at operation. It is not surprising that the pathologist rather than the clinician has taken a special interest in these "nodules." Their variety and relatively high frequency at necropsies have given rise to a number of theories pertaining to etiopathogenesis and possible clinical importance. Of interest, naturally, is the question as to what hamartomas in general represent and as to what eventually may become of them.

In Virchow's[34] opinion, cortical fibromas represented the end-stage of a focal interstitial nephritis (nephritis interstitialis tuberosa). His theory was supported by Lubarsch,[21] who emphasized their inflammatory nature by pointing out the fact that "these nodules nearly always show adhesions to the renal capsule." Stimulated by the new concept of Albrecht, Genewein[11] studied a number of medullary nodules theretofore called fibromas and was able to demonstrate their hamartomatous nature by the presence of tissue elements other than fibroblasts and connective tissue, namely, residual renal tubules. At the same time he pointed out that the biologic behavior was different from that of true tumors: hamartomas are characterized by a slow development, nonin-

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vasiveness, morphologic similarity of all components to those of the matrix, and the fact that the growths may become temporarily stationary.

Zangemeister [40] found 114 such typical medullary renal fibromas at necropsy in 110 patients, most of them in people past the age of 50 years. His series showed no predominance in one sex.

Mixed Mesenchymal Hamartoma.—The renal mesenchyme is the tissue of origin of a number of true tumors, such as angiomas, lipomas, leiomyomas, and their malignant variants. These tumors represent neoplastic growths of blood vessels, of fat tissue, or of smooth muscle. All three morphologic elements are found in the grossly similar mixed mesenchymal renal hamartoma. For the latter a variety of terms, such as lipomyohemangioma, angiomyolipoma, or lipoma teleangiectodes renum, has been used merely to describe the components and their respective predominance in the microscopic picture.

It is beyond the scope of this paper to elaborate on the hypotheses pertaining to the derivation of smooth muscle and adipose tissue in renal tumors and hamartomas. Ulm [33] notes that controversial hypotheses for the origin of smooth muscle have been proposed by Borst [5] (renal capsule, blood vessels, and renal pelvis of the fully developed kidney), Wilms [39] (displaced undifferentiated embryonal tissue), Meyer [25] (mesodermal cells of the myotome), Lubarsch [22] (renal capsule), Hess [14] (arterial wall), and Inglis [16] (neural crest). A generally accepted hypothesis is that the lipomatous component is derived from the renal capsule, although one may consider heteroplasia of connective tissue or embryonal inclusion of adipose tissue in the kidney.

Renal hamartomas of the angiomyolipomatous type occur relatively frequently in patients with tuberous sclerosis. The latter is recognized as an entity that comprises the following features: mental retardation, epilepsy, adenoma sebaceum, phacoma of the retina, multiple mixed tumors of the kidney (hamartomas), and a familial history of tuberous sclerosis. The same tendency to produce multiple tumors is found in another systemic disease, namely, in neurofibromatosis. Moolten,[26] in his most comprehensive and informative study of the tuberous sclerosis complex, advanced the concept that "the similarity between the tuberous sclerosis complex, multiple neurofibromatosis (von Recklinghausen), encephalotrigeminal angiomatosis (Krabbe), Hippel-Lindau's disease and related syndromes is sufficient to establish them all as forms of disseminated hamartosis."

The renal hamartoma occurring in patients without manifestations of tuberous sclerosis is histologically indistinguishable from the renal hamartoma occurring in patients having the other lesions of the tuberous sclerosis complex. Moreover, a reasonable explanation has yet to be found for the occurrence of solitary predominantly large hamartomas in patients without tuberous sclerosis and of multiple usually small hamartomas in patients with tuberous sclerosis. We have recently observed four patients with renal hamartoma not affiliated with tuberous sclerosis.

Report of Cases

CASE 1.—A 42-year-old woman was first examined here in June, 1954, because for five months she had had repeated episodes of severe pain in the left flank, concomitant with chills and fever. She had been hospitalized elsewhere after the second episode, in January, and the diagnosis of polycystic renal disease was established at that time.

The family history was noncontributory; there was no evidence of polycystic renal disease in her family. Physical examination disclosed a well-developed woman in no acute distress. The temperature was normal, and the blood pressure was 116/70 mm. Hg. Both kidneys were enlarged and tender on palpation. The examination revealed no signs of tuberous sclerosis. The laboratory findings were within normal limits except a culture of the urine which was positive for Alcaligenes faecalis.

The urologic examination included an intravenous urogram, which disclosed a lobulated enlargement of both kidneys, a reduced renal function evidenced by delayed excretion and diminished concentration of the contrast medium, dilatation of both renal pelves, and an elongation of all calyces. The

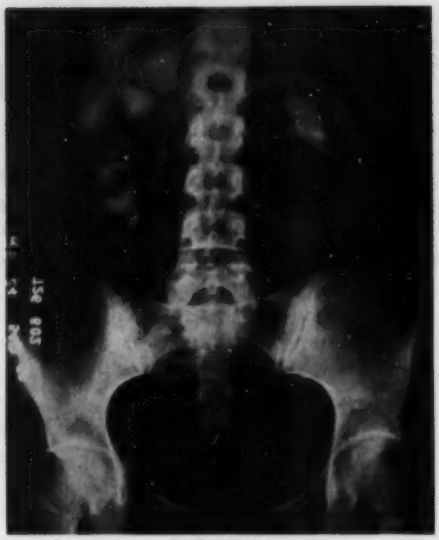


Fig. 1.—Intravenous urogram showing large renal masses; one hour after injection, contrast medium has accumulated in dilated calyces in a manner suggestive of polycystic disease.

tentative clinical diagnosis was bilateral polycystic disease (Fig. 1).

The patient was given ambulatory treatment with sulfonamides and was kept under clinical observation. Although mild symptoms of urinary tract infection persisted, the patient felt well until November, 1956, when she experienced another episode of severe pain in the left flank. When she returned for reexamination, in May, 1957, the right kidney was considerably larger than in 1954. A retrograde pyelogram confirmed the progressive enlargement and disclosed multiple pressure deformities of the pelviocalyceal system bilaterally.

The patient was admitted to the hospital for an exploratory operation. The greatly enlarged right kidney extended over the brim of the pelvis, and its surface appeared irregular because of diffuse tumorous masses that surrounded fluctuant, cystic areas, especially at the upper pole. A biopsy specimen was obtained from the relatively firm middle portion of the renal mass, and clear fluid was aspirated from several large cysts. Subsequent exploration of the left kidney revealed a pedunculated, firm tumor at the lower pole, approximately 10 cm. in diameter. The left kidney proper was enlarged; the surface appeared irregular, and multiple, fluctuant cysts could be palpated through the cortex. In view of the massive involvement of both kidneys, further surgical treatment was abandoned.

Pathologic Findings.—Gross

The wedge-shaped biopsy specimen of the right kidney measured 3×2×2 cm. and was uniformly yellowish tan. It was somewhat firmer than normal perirenal adipose fat tissue and showed no distinct structures.

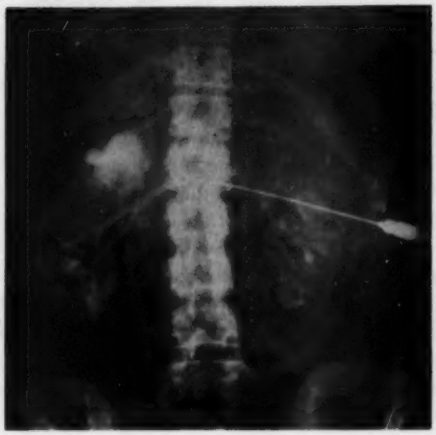
Microscopic

Multiple sections through the specimen showed predominantly mature fat cells, with intermingled well-differentiated smooth muscle. The highly vascular areas were characterized by innumerable, moderately thickened, tortuous blood vessels, surrounded by sheets or scattered bands of smooth muscle and fat cells. Many smooth muscle cells had vesicular nuclei, but mitosis was not seen. Adjacent to a cystic structure lined by urothelium were patches of smooth muscle similar to those within the adipose tissue. Renal parenchyma was absent in all of the sections. The pathologic diagnosis was angiomyolipoma (hamartoma).

Subsequent Course

The postoperative course was uneventful and the patient left the hospital on the eighth postoperative day. Two months later she had to be hospitalized elsewhere because of severe pain in the left flank and mild pain in the right flank. These episodes of recurrent pain and the progressive enlargement of both kidneys warranted consideration of a partial removal of the angiomyolipomas. The patient was readmitted here on Feb. 23, 1958, for surgery. A preoperative renal angiogram disclosed highly vascular areas, especially at the lower pole of the left and the right kidney, puddling of contrast medium, and distorted

Fig. 2.—Translumbar aortogram. The elongated and enlarged arteries terminate in irregular vascular pools in the lower half of each kidney.



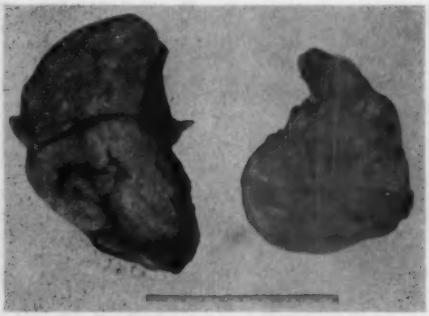


Fig. 3.—Cross section of the surgical specimen (left and right) showing smooth, slightly bulging cut surfaces. Triangular area in lower half of the specimen on the right represents central necrosis which was revealed by pooling of contrast medium in renal angiogram.

blood vessels (Fig. 2). By a transperitoneal approach, the anterior surface of the left kidney was exposed first. A somewhat lobulated ovoid mass, measuring about 15 cm. in diameter, at the lower pole of the kidney was found to be attached to the kidney proper by a wide bridge of tumorous tissue. The appearance of the left kidney was comparable with that at the previous laparotomy. The ovoid mass was removed by sharp transection of the bridge immediately at the lower pole and blunt dissection of the mass itself. The right kidney appeared slightly larger than at the time of laparotomy, and the lower one-third was somewhat firmer that the cystic upper two-thirds. In the absence of any distinct cleavage plane, the reduction of the large mass could be achieved only by sharp transection of the kidney at the level of the lower third. The transected lower calyces were closed with several stitches.

The postoperative course was uneventful, and the patient was discharged on the 10th postoperative day. She has remained asymptomatic up to the date of this report.

Second Pathologic Report.—Gross (Fig. 3)

The right specimen measured 15×9×7 cm. and weighed 420 gm. It consisted of a roughly ovoid mass of tumorous tissue, apparently encapsulated and partially divided into various-sized pseudolobules. The capsule showed focal hemorrhages. The cut surface was dark ocher and rubbery and showed focal necrosis in many areas, one of them 2 cm. in diameter. Medium-sized vascular channels were seen on several sections through the specimen.

The left specimen measured 13×8×4 cm., weighed 300 gm., and was externally similar to the right specimen, except for a homogeneous rather than a lobulated appearance.

Microscopic (Figs. 4A, 4B, and 4C)

Sections from the left and right side were identical in morphologic appearance and compared well with the microscopic picture

Fig. 4A.—Photomicrograph showing prominent blood vessels of various calibers within hamartoma, formed predominantly by fat tissue, and hypercellular areas representing smooth muscle. Hematoxylin and eosin; ×15.

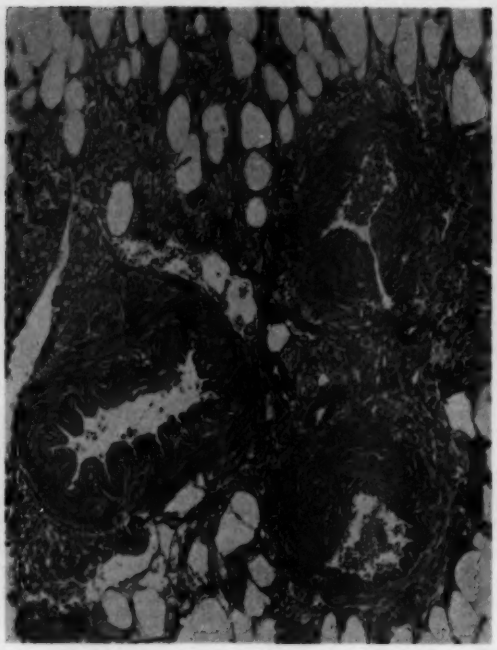


Fig. 4B.—Photomicrograph showing thick-walled blood vessels with collar-like structures of spindle cells (smooth muscle) and typical radiation of the latter into the fat tissue component. Hematoxylin and eosin; × 100.

of the biopsy specimen taken at the previous exploratory laparotomy. There were tortuous blood vessels of varying calibers, with smooth muscle walls from which bundles of smooth-muscle fibers irregularly radiated into the surrounding fat tissue. Fresh focal

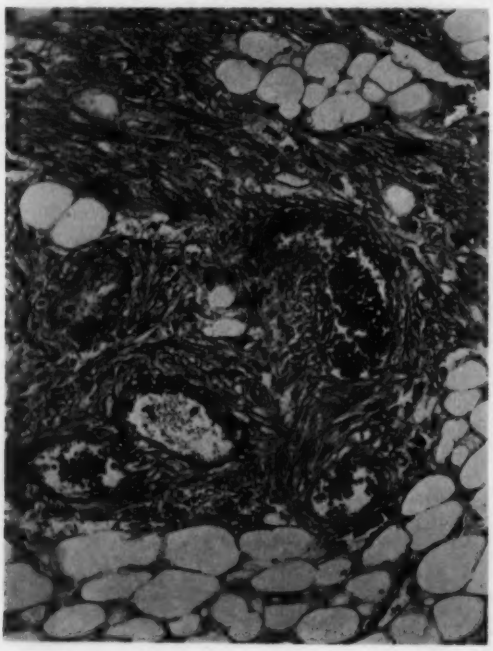


Fig. 4C.—Photomicrograph showing blood vessels, spindle cells with coarse fibrils (smooth muscle), and fat cells. Phosphotungstic acid-hematoxylin; × 160.

hemorrhages and focal necrosis were present, predominantly within the highly vascular areas. Renal tissue was not found in any specimen.

Pathologic diagnosis was angiomyolipoma of the left and right kidney; focal necroses, and fresh hemorrhages.

CASE 2.—A 59-year-old woman was first examined here in January, 1955, because of longstanding "stomach trouble." She had had midepigastric soreness, cramps, burning, and gas pain for at least one year, and constipation for several years.

The family history was noncontributory. The patient had undergone a pelvic operation in 1919, an appendectomy in 1928, and a cholecystectomy in 1929; she had a "heart attack" in 1949.

Physical examination revealed an obese woman, weighing 196 lb., in no acute distress. The temperature was normal; the blood pressure was 150/80 mm. Hg. A firm, tender mass, approximately 20 cm. in diameter, was palpable to the right of the umbilicus. The urinalysis revealed no pathologic findings. There was no evidence of tuberous sclerosis.

A diagnosis of cyst of the right kidney was made, and the patient was admitted to the hospital. Intravenous urography disclosed normal renal function and normal pelviocalyceal systems, but there was a slight lateral displacement of the lower pole of the right kidney. A renal angiogram showed an irregular, somewhat mottled collection of contrast medium in the region adjacent to the lower pole of the right kidney. These findings were strongly suggestive of tumor of the lower pole of the right kidney, and surgical treatment was advised. At operation it was found that the lower third of the right kidney had been replaced by a large tumor, about 20 cm. in diameter. The mass was yellow and rather soft, apparently because of central necrosis, and in some areas was separable from the kidney proper. A total right nephrectomy was performed.

The postoperative course was uneventful. The patient was discharged on the 11th postoperative day; repeated examinations since have revealed no recurrence.

Pathologic Findings.—Gross

The specimen measured 20×16×8 cm. and weighed 950 gm. The right kidney with 10 cm. of ureter was incorporated in the tumorous mass. A flimsy encapsulation seemed to be present; the kidney could be separated from the mass everywhere except in the region of the pelvis and the lower pole. Externally the mass was a mottled red and golden yellow. On cross section the tumorous mass was somewhat firmer than the usual perirenal fat and slightly more homogeneous.

Microscopic

Multiple sections through the mass revealed typical mature adipose cells, with islands of increased vascularity in angiomatous arrangement. The majority of blood vessels were characterized by thick walls, with collar-like structures of smooth muscle. In several areas bands of spindle-shaped cells and smooth muscle radiated into the surrounding adipose tissue, thus forming septum-like structures within the adipose tissue. The transition from the mass to the renal parenchyma was immediate. There was no evidence of capsule formation. In some areas, adipose tissue extended between the renal tubules immediately bordering the mass. There was no evidence of atrophy from pressure or invasion of the renal parenchyma. Phosphotungstic acid-hematoxylin stains disclosed coarse fibrils in the spindle-shaped cells.

Pathologic diagnosis was angiomyolipoma (hamartoma).

CASE 3.—A 24-year-old man was first examined here, in 1940, because of painless hematuria. A cystoscopic examination and retrograde pyelogram disclosed no pathologic findings. The hematuria was believed to be caused by a deficiency of ascorbic acid (vitamin C).

From 1940 until May, 1957, the patient was examined on eight occasions because of recurring painless hematuria. The symptom-free intervals varied from several months to three years; the episodes of hematuria lasted from a few days to three months. Only occasionally had he noticed concomitant pain in the right flank. The lower urinary tract was normal on all cystoscopic examinations, but each time blood issued from the right ureteral orifice. A retrograde pyelogram, made in 1949, showed a small filling defect in the right lower calyx group, but this was questionable three months later. In 1954, a definite deformity of the right lower calyx was interpreted as a pyelonephritic change.

After another episode of gross hematuria, this time with considerable pain in the right flank, the patient was admitted to the hospital, in May 1957. The cystoscopic findings were negative, but the small deformity of the right lower calyx was now considered to be diagnostic of a slowly growing lesion, such as an angiomatous hamartoma, and operation was advised.

The lower third of the right kidney was resected without difficulties on May 16, 1957. The post-

operative course was uneventful, and the patient was discharged on the 15th postoperative day. Follow-up examinations revealed no hematuria since operation. The patient had no signs of tuberous sclerosis.

Pathologic Findings.—Gross

The resected portion of the kidney appeared normal as to external surface, color, and consistency. On cross section a small, circumscribed, spongy, brownish red lesion about 1 cm. in diameter was found immediately adjacent to one of the calyces.

Microscopic

Close to a renal papilla and in part lined by urothelium there was an ovoid nodule approximately 1 cm. in diameter composed of large, dilated, thin-walled blood vessels, numerous smaller thick-walled blood vessels, adipose tissue, and smooth muscle. The large dilated blood vessels were filled with erythrocytes, especially in one region immediately bordering the renal parenchyma. Broad strands of mature smooth-muscle cells radiated from the circular layers of the thick-walled arteries into the surrounding adipose tissue with which they seemed to form an irregular network. There were also islands of loose connective tissue with numerous erythrocytes, representing fresh hemorrhage into the growth. There was no evidence of encapsulation. The renal parenchyma immediately adjacent to the growth appeared normal, except for a mild vacuolization of the tubular epithelium within the border zone.

Pathologic diagnosis was angiomyolipoma (hamartoma).

Case 4.—A 56-year-old woman was first examined, in May, 1947, because of pain in the right lumbar region and urinary frequency. Six years previously she had been injured in an automobile accident. There was no evidence of a back injury, although pain in the right lumbar region had been present constantly and had become severer during the last two years. She had arthritis in the right shoulder and the fingers of the right hand. A repair of a cystocele and a pelvic operation had been performed 15 years ago.

Physical findings were moderate cystocele, large rectocele, exogenous obesity, and a palpable and ballottable, though not tender, right kidney. The orthopedic findings were a moderate hypertrophic arthritis, with considerable narrowing of the third and fourth lumbosacral interspaces. There was no evidence of tuberous sclerosis.

Cystoscopic examination disclosed a mild trigonitis. A bilateral retrograde pyelogram was normal; the urine culture was sterile. The patient was advised to have the rectocele surgically repaired and to return for reexamination of the upper urinary tract.

In April, 1948, the patient was admitted to a hospital elsewhere because of acute pain in the right flank, radiating to the right groin, and fever. She was again examined here, in June, 1948. At that time the right kidney did not seem larger on palpation than in 1947, but an intravenous urogram showed some flattening of the right renal pelvis, with an impression deformity and irregular calyces suggestive of tumor within the upper half of the right kidney. A right nephrectomy was performed.

The postoperative course was uncomplicated, and the patient left the hospital on the 12th postoperative day. Although the patient did not return for a follow-up examination, we have been notified that she is currently in good health.

Pathologic Findings.—Gross

The kidney measured 14×9×6 cm. and weighed 365 gm., including a small amount of perirenal adipose tissue. An ovoid, slightly bulging mass, approximately 7 cm. in diameter and somewhat firmer than the remainder of the renal substance, comprised the upper three-fifths of the kidney. The external surface of the mass was smooth and covered with a transparent, tough membrane. On cross section, the cut surface was yellow and greasy and scattered throughout were irregular regions of gray, fleshy tissue up to 1 cm. in diameter. The remainder of the kidney had typical corticopyramidal architecture. There was a fairly distinct demarcation of the growth but no evidence of encapsulation.

Microscopic

Multiple sections disclosed a nonencapsulated growth composed of mature adipose tissue, smooth muscle, and numerous thick-walled blood vessels. Areas of adipose tissue were intermingled with sheets of smooth muscle and markedly vacuolized and polyhedral cells. The highly vascular areas comprised considerably thickened, tortuous, blood vessels with narrowed lumens. Most of the vascular walls lacked the normal architecture and appeared rather homogeneous and hyalinized. Some of them exhibited considerable intimal thickening and folding. The majority of vessels were surrounded by collar-like structures of smooth-muscle cells that partly formed sheets extending

into the adipose tissue. In one region there was evidence of old and fresh hemorrhages, characterized by many erythrocytes and a massive depot of hemosiderin. Extension of adipose tissue between slightly dilated renal tubules at the border zones was noted.

The original diagnosis was well-differentiated liposarcoma because of the renal infiltration and the fact that the tissue originally sectioned did not contain the characteristic angiomatous areas with thick-walled blood vessels. Reviewing the original and studying the additional sections led to the correct pathologic diagnosis of angiomyolipoma (hamartoma).

Comment on Cases

The four cases presented here have little more in common than the microscopic appearance of the renal lesions and the fact

TABLE 1.—*Summary of Previously Reported Cases*

Authors	Year	Sex	Age	Clinical Symptoms	Pathologic Findings	Pathologic Diagnosis
Apitz [6]	1943	Not stated		Not stated	Necropsy findings	3 cases of angiomyolipoma
Brody & Lipshutz [7]	1955	F	29	Large abdominal mass 10 yr. after removal of leiomyolipoma on left side, asymptomatic	1/3 kidney replaced by masses; largest 7×5 cm.; separate pararenal mass 17×7×5 cm.	Intrarenal & pararenal angiomyolipoma
Heckel & Penick [10]	1948	F	47	Acute flank pain, no hematuria	Tumor 8×4×4 cm., with cystic degeneration	Mixed tumor, lipomyohemangioma
Hulse & Palik [11]	1951	F	59	Chills, fever, pyuria	Irregular nodule in midportion of right kidney, 3 cm. diameter	Hamartoma (angiomyolipoma)
Le Brun, Kellett, & Macalister [12]	1955	F	29	Severe pain, abdominal tumor, hemorrhage, shock	Tumor, 10×6×11 cm. replacing ¾ of kidney	Angiomyolipoma
Morgan, Straumfjord, & Hall [13]	1951	F	70	Severe abdominal pain, nausea, tumor right upper quadrant, no hematuria	Hollowed-out area in right kidney containing soft-tissue masses, ruptured aneurysm of renal artery	Angiomyolipoma
Rusche [14]	1952	F	23	Right flank pain 12 hr. postpartum, palpitation, mass right upper quadrant, hematuria	Tumor replacing lower half of right kidney, necrosis, & hemorrhage	Angiomyolipoma
		M	50	Severe abdominal pain, fever, patient semicomatose	Incidental necropsy finding: multiple nodules 0.7-1.5 cm. in left kidney	Angiomyolipoma
		F	76	Severe abdominal pain, nausea, myocardial failure	Tumor at lower pole left kidney, 4 cm. in diameter	Angiomyolipoma
Taylor & Genters [15]	1958	F	40	Severe left flank pain, no hematuria	Large necrotic tumor	Angiomyolipoma
Tweeddale, et al. [16]	1955	F	27	Acute left flank pain, microscopic hematuria	Tumors 9×6 cm., 5×4 cm. at upper pole left kidney, bland thrombus renal vein	Angiolipoleiomyoma
		F	25	Not stated	Tumor at lower pole right kidney, 6 cm. in diameter	Angiolipoleiomyoma
Weaver & Carlquist [18]	1957	F	12	Acute right flank pain, hematuria	Tumor 2 times size of right kidney	Angiomyolipoma
Present authors	1958	F	42	Severe left flank pain, chills & fever, known bilateral polycystic disease, no hematuria	Massive involvement of both kidneys by hamartoma	Angiomyolipoma
		F	59	Midepigastric soreness, cramps, burning, & gas pain, palpable mass 20 cm. diameter in rt. hypochondrium, no hematuria	Large hamartoma 20×16×8 cm. weighing 950 gm. involving rt. kidney	Angiomyolipoma
		M	24	Recurrent painless hematuria over 17 yr., considerable right flank pain on admission	Small hamartoma 1 cm. diameter adjacent to lower calyx of right kidney	Angiomyolipoma
		F	56	Chronic right flank pain, with 1 episode of acute exacerbation, urinary frequency, no hematuria	Hamartoma 7 cm. diameter in upper half of right kidney	Angiomyolipoma

that all four patients were adults without lesions of the tuberous sclerosis complex. The diameters of the hamartomas ranged from 1 to 20 cm. In three patients the hamartoma was unilateral, and in one patient it was bilateral. The varying duration of symptoms is especially remarkable because the smallest hamartoma was supposedly present for 17 years, while the largest hamartoma, involving both kidneys, caused symptoms for only six months. The variable findings in these four cases certainly do not offer a sound basis for clinicopathologic studies of renal angiomyolipoma without a review of other reported cases and, equally important, of the general concept of this peculiar lesion.

A review of the literature revealed no more than 15 cases of renal angiomyolipoma in which the diagnosis was clearly established and in which there was no evidence of tuberous sclerosis. The pertinent data of 12 cases and our additional 4 cases are presented in Table 1. The three cases of Apitz[2] were reported without details and will not be included in the discussion of the clinicopathologic aspects.

Two facts will be noticed at once: first, all but one of the patients were adults, the exception a 12-year-old child; second, there was a definite predominance of women; of 16 patients, 14 were women.

Symptoms.—Hematuria seems to be an unreliable symptom, occurring in only four patients; in two the hematuria was gross, and in two it was microscopic. It is surprising to see that very large hamartomas caused neither gross nor microscopic hematuria, while one small hamartoma, only 1 cm. in diameter, caused multiple episodes of gross hematuria over a period of 17 years. In the latter case, however, hematuria served as a diagnostic sign for angiomatous hamartoma, since it occurred as multiple episodes over many years and in the presence of roentgen evidence of an extremely slowly growing lesion. The relatively low incidence of hematuria can be explained by the fact that hamartoma does not destroy the renal parenchyma but grows by expansion, generally resulting in mere compression of the renal tissue. Hematuria apparently occurs only as a sequel of massive hemorrhage into the hamartoma, with resulting trauma to the renal parenchyma, or, as in one of our cases, because of the proximity of the hamartoma to the mucosa of the renal pelvis.

Flank pain occurred in 13 of the 16 patients, usually in an acute severe form and less commonly as an acute exacerbation of a chronic dull pain. Both acute severe pain and acute exacerbation of chronic pain indicated intrarenal hemorrhage or hemorrhage into the perirenal adipose tissue, leading to hemorrhagic shock in only one reported case.

Fever was observed in 3 of 16 patients.

Treatment.—Nephrectomy was successfully carried out in 11 patients. Of the remaining five patients, one underwent resection of only the lower pole; one underwent an operation for bilateral partial resection of an extensive hamartoma involving bilateral polycystic kidneys, and two died without surgical intervention within one week; the treatment was not stated in one case.

Pathologic Diagnosis.—The diagnosis of angiomyolipoma was supported in all of the reported cases by detailed description of the microscopic findings and by photomicrographs, all of which displayed the typical morphologic elements. The characteristic features were recognized by us and described as follows: an increased number of thick-walled, often tortuous, blood vessels, frequently in angiomatous arrangement, surrounded by collars of smooth-muscle cells which extended as strands or septum-like structures into the adipose tissue. Both adipose tissue and smooth muscle were found in the mature form. In none of the descriptions was a tumor capsule mentioned. The hamartoma either was immediately adjacent to normal renal parenchyma or bordered a zone of renal parenchyma that exhibited a slight degree of pressure atrophy. Cystic degeneration was observed in but a few cases, in some of which the

TABLE 2.—*Tumor in Polycystic Kidneys Reported in Literature*

Yr. of Report	Authors	Type of Tumor
1928	Weiser [27]	Hypernephroma
1934	Walters and Braasch [25]	Adenocarcinoma & 2 tumors (not mentioned in detail)
1936	Wells [24]	Intracystic papilloma
1937	Tomoff [21]	Hypernephroma
1940	Mellcow and Gile [14]	Hypernephroma
1943	Bobbitt [4]	Hypernephroma
1945	Lowsley and Curtis [10]	Angiomyosarcoma, fibrosarcoma
1946	Hayward [13]	Hypernephroma
1953	Johnson [17]	Carcinoma
1954	Mathé [29]	Cholesteatoma

cysts were either lined by urothelium or the result of necrosis.

Hamartoma and Polycystic Disease of the Kidney.—The most interesting of our four patients is the woman who was originally believed to have bilateral polycystic disease and was later found to have extensive bilateral hamartoma with polycystic disease of the kidneys (Case 1). We have been unable to find a similar case reported in the literature. However, simultaneous occurrence of polycystic disease and other types of tumor has been reported in 13 cases (Table 2). Polycystic kidneys per se have been considered to constitute an example of hamartoma [6,18] or hamartoblastoma.[28] This hypothesis may be acceptable if we use the term "hamartoma" in a broad way for any "error in development." In our case, then, we would be concerned with an error in development in two directions, namely, polycystic kidneys and angiomyolipoma. The therapeutic problems involved are obvious and will not be discussed.

Comment

The rare occurrence of renal hamartomas would not warrant a lengthy discussion of histopathogenesis or classification if we were absolutely certain that angiomyolipomas are merely benign developmental errors and do not undergo malignant changes. Is there any evidence for such malignant changes? The possibility cannot be denied. On the other hand, one may argue that so-called malignant angiomyolipomas, such as those reported by Burkitt [8] and Berg,[3] had been malignant mixed mesenchymomas a priori. In the case of a woman, 62 years of age, described by Burkitt, multiple small secondary nodules in the liver were present in addition to an extensive primary renal tumor. Both primary and secondary tumors were composed of mature adipose tissue and masses of smooth muscle cells arising from or in the vicinity of moderate-sized blood vessels. The nuclei showed pleomorphism but no mitosis. Berg observed a renal angiolipomyosarcoma (malignant hamartomatous angiomyolipoma) in a 59-year-old woman who died of carcinoma of the lung. The renal tumor had invaded the renal vein, thus providing evidence for the malignant nature of the hamartomatous lesion "contrary to other reports lacking either evidence for the malignancy of the hamartomatous lesion or evidence for the hamartomatous nature of the malignancy."

It can be assumed that a fair number of malignant mixed mesenchymomas reported in the literature could be interpreted as malignant hamartomatous angiomyolipomas on the basis of their morphologic composition. But such an interpretation is speculative, because it is a retrospective assumption postulating that the presently malignant tumor originated in a previously benign hamartoma. The link between these two still has to be found, namely, a histopathologically confirmed angiomyolipomatous hamartoma which after a certain time interval underwent malignant changes. Neither Berg nor Burkitt can provide the evidence. We, therefore, must conclude that the definition of a renal angiomyolipoma as a hamartoma still stands. The angiomyolipoma is an error in development and to our present knowledge is not a true tumor apt to undergo malignant changes.

Summary

Four cases of renal hamartomas of the angiomyolipomatous type are reported. Three occurred in women; one, in a man. None of the patients exhibited signs of tuberous sclerosis. In one case a massive

involvement of both kidneys concomitant with bilateral polycystic disease posed several therapeutic problems. In the second case there was an unusually large hamartoma at the lower pole of the right kidney. The third case was characterized by episodes of hematuria over 17 years, without any other signs and symptoms. A small filling defect of the lower calyx was recognized roentgenographically as evidence of a slowly growing lesion, and a resection of the lower pole of the right kidney was performed. The angiomyolipoma measured only 1 cm. in diameter. In the fourth case, acute pain in the right flank, a palpable tumor of the right kidney, and a filling defect, demonstrated by intravenous urography, were the indications for nephrectomy. The original diagnosis in this case was liposarcoma, but additional sections led to the correct diagnosis, hamartoma of the angiomyolipomatous type.

Fifteen cases of angiomyolipoma reviewed from published reports are discussed.

Renal angiomyolipomas are hamartomas that rarely occur in patients without lesions of the tuberous sclerosis complex and then are unifocal within the kidney. Histopathologically, they do not differ from the usually multiple renal hamartomas in patients with tuberous sclerosis. In contrast to the small medullary fibromas, angiomyolipomas may reach a considerable size and produce the clinical symptoms of renal tumors, namely, pain, fever, and hematuria. They may, on the other hand, cause no symptoms at all. Hematuria is indicative of an angiomyolipomatous hamartoma only in patients with both chronic recurrent episodes and roentgenologic evidence of a slowly growing lesion.

Angiomyolipomas are hamartomas, i. e., they are due to an error in development. So-called malignant angiomyolipomas, therefore, cannot be labeled "malignant hamartoma" but must be classified as malignant tumor of mixed mesenchymal origin (malignant mesenchymoma). No evidence has yet been found that malignant changes do occur in hamartomas of the angiomyolipomatous type.

Surgical removal of the hamartoma by either excision or nephrectomy is the only form of treatment. Immediate surgery is indicated in patients with acute symptoms of hemorrhage into tumor, kidney, or perirenal fat.

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Congenital Absence of the Parathyroid Glands

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Cases of insufficiency of the parathyroid glands not related to goiter surgery are clinically uncommon and still more seldom encountered at the autopsy table. Thus, in Steinberg and Waldron's comprehensive review of chronic idiopathic hypoparathyroidism, pathologic material was available in only 3 of the 52 cases discussed.[1] Well-documented reports of congenital absence of the glands, leading to prolonged infantile tetany and death, are equally rare and until now have been confined to the European literature.[2-4] The present case, that of a child who manifested a parathyroprivic picture throughout his two months of life, is offered as the fifth recorded example of congenital agenesis of the parathyroid glands.

Report of a Case

History

This full-term 2,920 gm. infant was delivered spontaneously from a 24-year-old Puerto Rican mother, whose prenatal course was complicated only by anemia. A female sibling, born 13 months previously, was in good health. Neuromuscular hyperirritability was noted at birth, and a serum calcium drawn at 12 hours was reported as 4.9 mg. %.

Physical Examination

After transfer to the pediatric service, the baby, at 39 hours of age, was described as a normally developed but "very jumpy" boy, who exhibited very jerky movements of his extremities when crying or disturbed. Deep tendon reflexes were hyperactive, and periodic clonus was noted. A positive Chvostek's sign (not unusual for this age) was elicited, but there was no carpopedal spasm or Trousseau's sign. The infant frequently emitted a peculiar high-pitched cry, characterized as "cerebral" by the attending pediatrician.

Course

The child had frequent episodes of tetany, manifested by generalized stiffness and shaking, slight cyanosis, and a high-pitched scream. Calcium chloride and calcium gluconate were administered per os, with poor initial response. Serum calcium at one week of age was reported as 6.0 mg. %, and phosphorus, as 8.6 mg. %. Total serum protein was 5.1 mg. % and remained within this range throughout the clinical course. Parathyroid hormone (Parathormone) administration (Ellsworth-Howard test) resulted in more than a twofold increase in urinary phosphate excretion, excluding lack of kidney end-organ response to parathyroid secretion as an etiologic factor. A roentgenological bone survey at age 2 weeks showed normal osseous density. ECG tracings reflected right ventricular dominance. Routine spinal fluid and urine determinations were unremarkable.

Addition of massive doses of vitamin D to the therapeutic regimen resulted in reversal of the abnormal Ca/P ratio; at 15 days of age, serum calcium was reported as 11.0 mg. %; phosphorus, as 5.1 mg. %, and alkaline phosphatase, as 6.5 Bodansky units. Oral calcium administration was discontinued, at one month of age, as the infant's neuromuscular hyperirritability diminished. Two tetanic seizures during the fifth week of life were controlled by calcium gluconate.

At 2 weeks of age, right upper lobe consolidation had developed, associated with copious mucopurulent nasal discharge. A bilateral pulmonary infiltrate gradually became apparent on x-ray, and the child grew progressively anemic and lethargic. At age 5 weeks, he began having bouts of diarrhea. Stool and nasopharyngeal cultures revealed no pathogenic organisms. Despite wide-spectrum antibiotic therapy the pulmonary infection progressed, leading to death 55 days after delivery.

Autopsy Findings

The pertinent postmortem findings were confined to the chest and neck organs. The thymus was totally absent from its usual location in the superior mediastinum. Since aberrant thymus has been described in many sites in the chest cavity and neck, some of them quite bizarre,[5] all suspicious tissue was preserved during a meticulous dissection of these regions. On microscopic examination, this proved to be lymphnodal in nature.

The only macroscopic abnormality of the cervical organs was agenesis of the thyroid isthmus. The neck block was removed and embedded *in toto*. Step sections, stained at intervals of 200μ, did not

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Fig. 1.—View, from above, of the great vessels, showing the esophagus and trachea enclosed in a ring formed by a right ascending aorta and arch, the surviving posterior portion of the left arch, a patent ductus arteriosus, and the pulmonary artery.

contain thymic or parathyroid tissue. Derivatives of branchial pouches I and II, namely, the middle ears, Eustachian tubes, and tonsillar fossae, were grossly intact.

Trachea and esophagus were encompassed but not compressed by a vascular ring formed in the following manner (Figure): The ascending aorta passed to the right of the trachea and at the level of the fourth dorsal vertebra turned to the left behind the esophagus to join the descending aorta. The left common carotid artery, right common carotid artery, and right subclavian artery arose in that order from the right aortic arch. The left subclavian artery originated at a 1 cm. diverticular structure which sprang from the junction of the right arch and the descending aorta. Between this outpouching (representing a remaining posterior portion of the left aortic arch) and the pulmonary artery ran a patent ductus arteriosus, which thus completed the ring. This vascular anomaly is comparable to Subgroup A3a in Edwards' classification of aortic arch malformations.[6]

The lungs were the seat of a patchy pneumonic process rich in lipid-laden macrophages, suggesting an aspirative basis. A Gridley stain for fungi was not contributory. Gram stains showed a mixed bacterial flora.

Since pituitary eosinophilia has been described in parathyroidectomized dogs,[7] as well as in Blaim and Lewicki's case of congenital parathyroid agenesis,[4] the hypophysis was subjected to a battery of differential stains. These failed to reveal an increase in eosinophils. A few small clusters of onkocytoid cells, similar to those occasionally encountered in pituitaries of all ages, took a modified Pearse-periodic acid-Schiff stain, suggesting that they were degenerating cells of the basophilic series.

Sections of the brain were examined with great care, as calcification of the basal ganglia has been reported in idiopathic hypoparathyroidism.[8] Several pallidal arterioles were partially surrounded by small, refractile coalesced globules and mulberry-like masses of pale-blue hyaline material, which did not take a Prussian blue or von Kossa stain. As these bodies are not uncommonly seen in infants dying of a variety of causes, their presence was felt not to be significant.

Final Anatomic Diagnosis

The final anatomic diagnosis was as follows: (1) Multiple congenital anomalies, including (*a*) agenesis of thymus, parathyroid glands, and thyroid isthmus, (*b*) double aortic arch with predominance of right arch (Edwards Subgroup A3a), and persistent patent ductus arteriosus; (2) hypoparathyroidism (clinical); (3) lobular pneumonia, bilateral; (4) acute splenic tumor.

Comment

There is little doubt in my mind that this is a case of congenital absence of the parathyroid glands. Conditions which can lead to hypocalcemia and tetany—chronic intestinal dysfunction, renal insufficiency, and vitamin D deficiency—were not present. The two-month duration of symptomatology is not compatible with the ephemeral "tetany of the newborn." Step sections of the neck organs did not reveal parathyroid tissue. The thymus, which develops with the parathyroids from branchial pouches III and IV, was absent. In addition, two other congenital malformations, agenesis of the thyroid isthmus and an anomalous vascular ring, were found.

Certain similarities between this and previously reported examples of parathyroid agenesis are apparent when the accompanying Table is examined. All cases

Collected Cases of Congenital Absence of the Parathyroid Glands

Authors	Year	Sex	Age Onset of Symptoms	Age Death	Thymic Abnormalities	Other Congenital Anomalies
Rössle	1932	M		9 hr.	Accessory lobe at level lower pole, right thyroid; persistent ductus thymopharyngeus	Acrania; agenesis right thyroid lobe; multiple cervical stalks & pits; branchiogenic cyst
Rössle	1932	F	6 wk.	10 wk.	Hypoplasia thoracic thymus; cord-like tissue in carotid sheath & behind left thyroid lobe	Agenesis thyroid isthmus; cleft uvula; heterotopic pancreas in small bowel
Rössle	1936	M	1 d.	12 wk.	Hypoplasia thoracic thymus; scattered small lobules along course of descent	Aniridia; absent macula lutea; malformed pinnae; reduplication right renal pelvis & upper ureteral segment; several accessory thyroid lobules at upper poles
Blaim & Lewicki	1955		8 wk.	5 mo.	None	Microcephaly; external hydrocephalus
Lobdell	1956	M	1 d.	2 mo.	Agenesis	Agenesis thyroid isthmus; double aortic arch with right predominance

Note: The 1927 case of Böttiger and Wernstedt [11] is not included, although probably belonging to the above group, since a single small parathyroid gland was found on microscopic examination.

except that of Blaim and Lewicki contained associated thymic abnormalities, ranging from hypoplasia to accessory or incompletely descended lobes in the neck. This is not surprising, since a local insult responsible for the failure of development of one primordium might be expected to affect also the future course of a second derivative of the same branchial pouch. Congenital defects of other organ systems were invariably present, in the majority more apparent clinically than the maldeveloped vasculature in the case at hand.

The paired fourth aortic arches, which run between branchial pouches III and IV, appear in normal embryos at approximately the same time as do the pouches, during the fourth week of intrauterine life.[9] Faulty formation of the left arch during this critical period would result in the anomaly of the great vessels noted in the present case. It is tempting, therefore, to assign responsibility for both the vascular malformation and the failure of development of the pouch derivatives to a single insult, acting at a time (fetal age, one month) when pouches and arches are beginning to form in the wall of the primitive pharynx. There is no clue to the nature of this deleterious force in either history or autopsy findings.

The premortem diagnosis of congenital absence of the parathyroid glands has been made, to my knowledge, only by Rössle,[3] who had the good fortune to encounter two examples at the autopsy table prior to his clinical feat. Rhyne and Carriker,[10] in reporting a living case of neonatal idiopathic hypoparathyroidism, postulated such an origin in their child and were probably correct, since it also suffered from bilateral congenital glaucoma. Certainly, prolonged hypocalcemia, hyperphosphatemia, and tetany in an infant with no evidence of rickets, renal disease, or intestinal dysfunction but with other demonstrable congenital anomalies should provoke a high index of suspicion.

Summary

A case is reported in which failure of development of the derivatives of branchial pouches III and IV resulted in infantile hypoparathyroidism. This increases the total number of recorded examples of congenital absence of the parathyroid glands to five.

The frequent association of this condition with anomalies of other organ systems is emphasized.

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Rupture of the Stomach in Children

Review of the Literature and a Report of Seven Cases

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Introduction

Spontaneous rupture of the stomach in children is a rare condition, having been found only 74 times in the English literature. We feel that a report of seven additional cases and a comprehensive review of the literature are of value at this time.

To Siebold goes the credit for first reporting, in 1825, a case of spontaneous gastric rupture in infancy.[1,2] Dunham and Goldstein[3] were able to find nine cases of spontaneous gastric rupture in the literature up to 1934, to which they added two new cases. Smythe,[4] reporting from this institution in the same year, added two cases of gastric perforation in newborn, premature infants, which were thought to represent rupture through acute peptic ulcers. Tow and Ross[5] added an additional case of spontaneous gastric rupture, in 1938. As in Smythe's cases, an acute gastric ulcer was thought to be the underlying etiology of the perforation.

Brody,[6] in 1940, reported the first case in which a congenital defect in the stomach wall was thought to be responsible for the perforation. A large gastric diverticulum which ruptured was present in this case. Herbut,[7] in his review of this subject, in 1943, was able to find 15 previously reported cases, 1 of which had been missed by Dunham and Goldstein[3] in their review. He added a case in which there was congenital absence of much of the gastric musculature. He believed this to have been the first case of spontaneous gastric rupture due to a congenital defect, being apparently unaware of Brody's earlier case.

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Pendergrass and Booth[8] reported a case in which neither an ulcer nor a congenital defect could be found. They attributed rupture in this case to birth trauma. This was also the second paper in which were mentioned two separate perforations found in the same stomach, the first having been reported by Smythe.[4] In 1947, Burnett and Halpert[9] reported another case of rupture due to congenital muscular weakness, in which other (unassociated) congenital anomalies were also present.

In this country, the first survival of a case of spontaneous gastric perforation in the newborn was reported, in 1951, by Kellogg and associates.[10] In their case rupture was thought to have been due to a peptic ulcer. In the same year, Ross, Hill, and Haas[11] reported a survival in one of their two cases. Ross and associates reported the first surviving case of rupture due to a congenital muscular defect. Legar, reporting in 1950, is said to have been the first to repair a spontaneous rupture, with survival.[1,2]

Several papers, dealing chiefly with solitary case reports, appeared from 1951 to 1955,[12-18] when Vargas et al.[1] summarized much of the literature and reported 11 new cases. These authors were able to find no less than 55 other cases in their review of the literature, bringing the total number of reported cases as of 1955 to 66. Several other papers appeared in 1955 and later,[2,19-25] bringing the known number of cases to date to 74. Several other cases are known to have been reported in foreign journals.

As late as 1954 it was stated, "Ruptures are found to occur in all portions of the stomach wall, having no one prominent

locality."[16] Meyer[25] correctly reports that the majority of spontaneous ruptures occur along the greater curvature.

Congenital anomalies, usually unrelated to the rupture, are frequently seen in these cases. There is, however, no constant type of anomaly reported.[1,3,4,6,9,10,14,15,17,21-23,25] Three of the reported cases have occurred in Mongoloids.[15,22]

Another frequently reported phenomenon is prematurity, as stressed by Vargas et al.[1] and first reported by Smythe.[4] Virtually all cases have occurred during the first two weeks of life.

Multiple etiologies have been suggested for the various cases of gastric rupture. Mann and associates[20] list these as (1) peptic ulcer, (2) injury due to gavage, (3) congestion due to septicemia and asphyxia, and (4) defects in the musculature of the stomach. It is likely that all of these have been responsible for some cases of rupture. Other causes proposed are rupture due to oxygen therapy[23] and birth trauma.[8,15,19]

Report of Cases

In order to determine the incidence of "spontaneous" gastric perforation, in the University of Tennessee, we examined the autopsy and hospital record room files for the past five years. From Aug. 1, 1953, to Aug. 1, 1958, there were a total of 5,160 autopsies performed, of which 1,778, approximately one-third, were in infants 2 months old or less. In the total autopsy group there were six cases of "spontaneous" gastric perforation, an incidence of 1:860 or 0.12%. Four of these five cases occurred in infants, giving an incidence of 1:444 or 0.25% of deaths in infancy (Table 1).

TABLE 1.—*"Spontaneous" Gastric Perforation in Children*

	Incidence
Autopsies	
All ages	5,160
2 mo. & less	1,778
Cases of gastric rupture	
Total	6
Less than 2 mo.	4
Incidence	
All age groups	1:860 (0.12%)
Incidence	
Children less than 2 mo.	1:444 (0.25%)

CASE 1.—This 9-month-old Negro girl was admitted to John Gaston Hospital on Nov. 30, 1953, with the chief complaints of irritability and vomiting of two days' duration.

Past history revealed that she was born at term after a normal pregnancy and labor. There were no serious illnesses prior to this admission.

At the time of admission examination a rectal temperature of 101.4 F was recorded. Her weight was 17 lb. 6 oz. Positive findings were limited to the abdomen, which was noted to be distended and slightly rigid. Bowel sounds were absent, and a fluid wave could be elicited. The clinical diagnosis of a ruptured viscus was substantiated by the finding of a massive pneumoperitoneum on x-ray.

An emergency laparotomy was performed a few hours after admission, at which time the abdomen was found to contain approximately 250 ml. of gas and fluid. A 1 cm. perforation along the lesser curvature on the posterior wall of the stomach was found. The edges were described as black and necrotic. This was partially excised and the wound repaired.

In spite of intensive supportive therapy the child died six hours after surgery.

The material excised at the time of surgery revealed necrotic mucosa and a marked acute inflammatory infiltrate. The muscle layers were present. A diagnosis of acute gastric ulcer was made (Fig. 1).

Blood cultures drawn during life were reported as having revealed no growth at the end of five days. No gavage tube or oxygen therapy was used prior to surgery.

At the time of autopsy, two hours after death, a purulent peritonitis was found. The operative repair of the gastric perforation was intact. In addition, another acute ulcer, located some 3 cm. distal to the repaired wound, was found. The remainder of the organs were within normal limits. A careful examination of the brain, both grossly and microscopically, revealed no abnormalities. Cultures of the peritoneal exudate revealed Streptococcus faecalis.

Microscopically, no muscular defects could be found in the stomach wall. The ulcer present was identical to that described in the surgical pathology report of the tissue removed during the operation.

No congenital malformations were present in this child.

CASE 2.—This premature Negro boy was born at John Gaston Hospital on Dec. 20, 1954, after

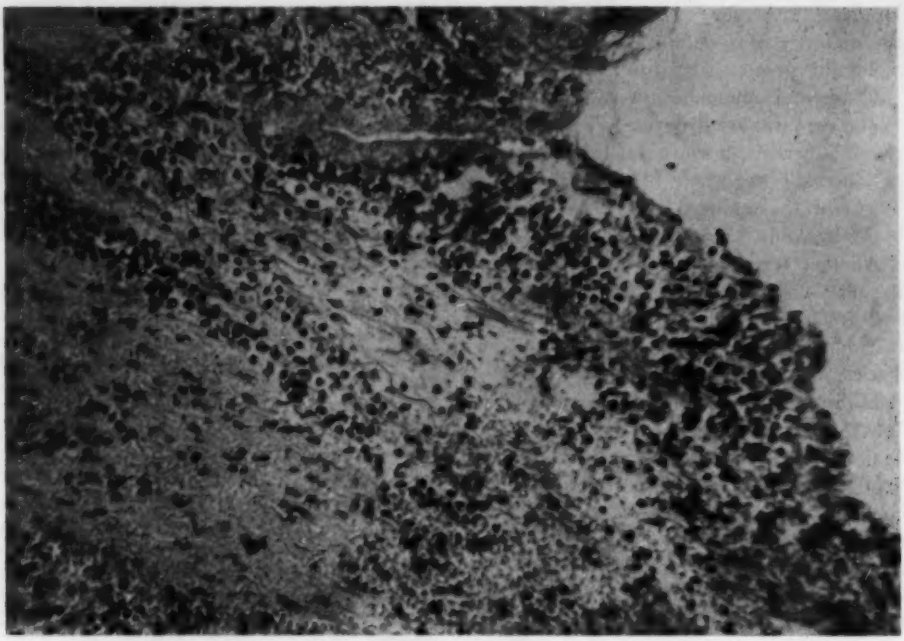

Fig. 1.—Photomicrograph from the edge of the acute gastric ulcer (Case 1). Hematoxylin and eosin; reduced 15% from mag. × 100.

30 weeks' gestation. Birth weight was 3 lb. 6 oz. The mother was a 44-year-old gravida 23, para 14, abortion 8, who experienced signs and symptoms of mild toxemia during pregnancy. Labor was described as short (two and one-forth hours), and delivery was spontaneous and unsterile. Cry and respirations were immediate.

On arrival at the premature nursery the child was placed in an incubator with oxygen and given prophylactic antibiotics. Gavage-tube feedings were instituted. Progress during the first five days of life was apparently normal. On Dec. 26, the child developed abdominal distention and began to vomit. An x-ray made on the following day revealed a massive pneumoperitoneum.

A laparotomy, performed as soon as the child could be prepared for surgery, revealed the abdomen to be filled with free air and bile-stained fluid. A 2 cm. serosal tear in the fundus, along the greater curvature, was found. A 5 mm. mucosal defect was present in this lesion. The defect was repaired and the infant returned to the nursery, where he died 11 hours later.

At the time of autopsy there was a severe generalized peritonitis present. The sutured area in the stomach was found to be intact. The remainder of the organs were not remarkable. No congenital malformations were present.

Microscopically, sections from the stomach revealed no congenital defects in the musculature. In the area of the rupture, mucosal autolysis and submucosal hemorrhage without a significant inflammatory response were present. The serosa was covered by fibrin and inflammatory cells.

Case 3.—This girl was born prematurely after 28-30 weeks' gestation to a young primigravida Negro. There were no maternal illnesses during pregnancy. Labor and delivery were reported as uneventful.

Primary respirations were slightly delayed, and oxygen by endotracheal catheter was administered. The child did well until the seventh day of life, when abdominal distention developed. The abdomen was noted to be tympanitic at that time. An x-ray taken shortly after the onset of abdominal distention revealed a massive pneumoperitoneum. The infant was immediately prepared for surgery.

At the time of laparotomy generalized purulent peritonitis was present. A 1 cm. perforation was present in the anterior wall of the fundus, 1 cm. from the cardioesophageal junction. The perforation was repaired, and the child, returned to the premature nursery, where she did well until she suddenly died, 46 hours after the operation.

At autopsy the gastric repair was noted to be intact, and a diffuse peritonitis was present. No congenital muscular defects could be found either grossly or microscopically.

Microscopic examination of the site of rupture revealed infiltration of the mucosa, submucosa, and muscularis by inflammatory cells.

CASE 4.—This Negro boy was born prematurely on April 9, 1955, in John Gaston Hospital. Birth weight was 2 lb. 4 oz. The child was cyanotic at birth, requiring endotracheal catheterization and suction. The infant was placed in an air lock and given oxygen. Gavage feedings were begun on the second day.

On the fourth day of life the child vomited a small amount of bright red blood. Shortly thereafter, and before an x-ray of the abdomen could be taken, the infant died. The clinical impression was a ruptured viscus.

At autopsy, a 1,000 gm., 38 cm. infant was observed. Generalized icterus with bright yellow pigmentation of the basal ganglia was present. There was a 5 mm. perforation in the anterior wall of the stomach.

Microscopically, no inflammatory cells or muscular defects were seen in the area of the perforation.

CASE 5.—This 6-year-old white boy was admitted to LeBonheur Children's Hospital, one of the teaching hospitals of the University of Tennessee, on Oct. 13, 1956, because of abdominal pain and distention.

Past history revealed four previous hospitalizations. He was known to have lipochondrodystrophy. On several previous occasions he had suffered from

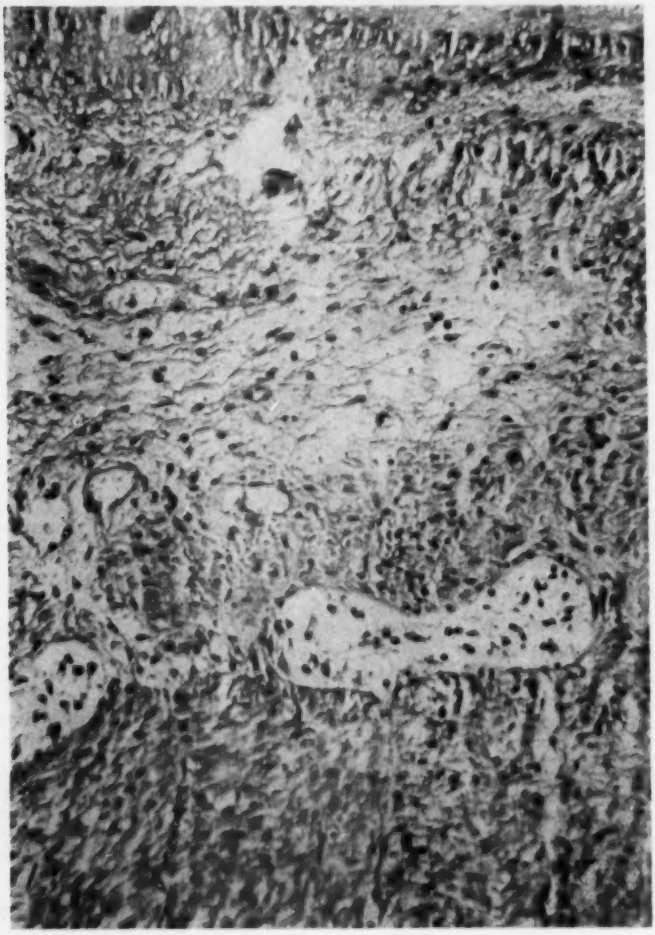

Fig. 2.—Degenerating cystic area in muscularis (Case 5). Hematoxylin and eosin; reduced about 10% from mag. × 100.

severe acute gastric dilatation, requiring gastric tube decompression.

On this last admission marked subcutaneous emphysema and cyanosis were present. Gastric intubation was attempted but was unsuccessful. He died shortly after admission in severe respiratory distress.

At autopsy, performed 30 mintes after death, marked subcutaneous emphysema was noted over most of the thorax, abdomen, and thighs. Intense cyanosis of the face and upper extremities was present. The stomach was greatly distended, containing over a liter of milky fluid. "A complete splitting of the greater curvature" of the stomach was noted. Approximately 500 ml. of milky fluid was present in the peritoneal cavity. A marked adynamic ileus was present. The lungs showed focal atelectasis. The remainder of the organs, except for some enlargement of the liver and spleen, were within the limits of normalcy.

Microscopic examination of the stomach revealed an absence of inflammatory cells about the site of rupture. The mucosa at the site of rupture was completely necrotic. There were cystic areas noted within the muscularis (Fig. 2). Sections from the brain and costochondral junctions revealed changes compatible with lipochondrodystrophy.

CASE 6.—This Negro infant was admitted to the hospital on Oct. 3, 1958, because of fever, mild diarrhea, and persistent crying. He was born at term after an uneventful labor. His diet had consisted of evaporated milk and unboiled well water.

Admission examination revealed a rectal temperature of 104 F, a tachycardia of 200 per minute, hepatomegaly, and paresis of the right leg. A left micro-ophthalmia was present. Moderate abdominal distention was present.

While he was in the hospital, blood and spinal fluid cultures were negative. Serum electrolytes were only slightly abnormal, with a carbon dioxide-combining power of 22.3 mEq. per liter and chlorides of 107 mEq. per liter. A white blood cell count revealed 9,950 wbc, with a slight left shift. A spinal fluid pleocytosis, with 38 wbc. per cubic millimeter, 36 of which were lymphocytes, was found. The spinal fluid protein was 90 mg. %, and the sugar, 46 mg. %.

Shortly after entering the hospital the patient's temperature fell to subnormal levels (96-97.5 F [rectal]), with the tachycardia persisting. The hepatomegaly decreased slightly. An x-ray of the chest revealed some consolidation, thought to represent early bronchopneumonia. No free air under the diaphragm was noted.

The child died after less than a day of hospitalization. At no time was a gastric tube inserted or oxygen therapy given.

At autopsy, three and one-half hours after death, a well-developed, 3,070 gm., Negro boy with a tense distended abdomen was observed. The left eye was noted to be small and was estimated to be only one-half to two-thirds as large as the right. The eyes were not removed for further examination. When the abdomen was opened, gas under pressure escaped through the incision. The abdomen contained approximately 25 mm. of cloudy fluid. The peritoneal surfaces of the intestines were hyperemic and covered by a thin layer of fibrinopurulent exudate. On examination of the stomach, a 2.5 cm. linear tear, extending from 1 cm. below the esophagus along the greater curvature, was found. There was no induration of the stomach wall about this perforation. The edges of the defect were thin and slightly blue in color. As the remainder of the gastrointestinal tract was opened, the only abnormalities encountered were hyperemia and superficial ulceration in the cecum and transverse colon.

The lungs were slightly heavier than normal and contained focal areas of consolidation. The heart and great vessels were normal. The spleen and liver were enlarged and acutely congested. The kidneys, ureters, and bladder were normal.

There was a 1.5 cm. area of depression over the posterior aspect of the left parietal lobe of the brain. There was slight thickening and yellow discoloration of the leptomeninges over this area. Small deposits of calcium were present in this area. On sectioning of the brain, multiple, small (1 to 3 mm.) areas of calcification and yellow discoloration were present in the basal ganglia and choroid plexuses of the lateral ventricles.

Microscopically, the findings in this case were those of peritonitis, early bronchopneumonia, colitis, acute congestion of liver and spleen, and congenital toxoplasmosis. Multiple sections of the stomach wall revealed no congenital abnormalities. No evidence of an acute peptic ulcer was found. A diffuse tissue basophilia was noted in the areas adjacent to the perforation (Fig. 3).

CASE 7.*—This premature (2,025 gm.) Negro boy, one of twins, was born on Dec. 12, 1956, after a normal pregnancy, labor, and delivery. No resuscitation was necessary. The child fed poorly, and on the third day of life feedings via gastric tube were begun. On the following day abdominal

* Dr. Richard Walker, of this department, gave me permission to use the material from this case, which he examined while serving as Chief, Laboratory Service, Memphis Naval Hospital.

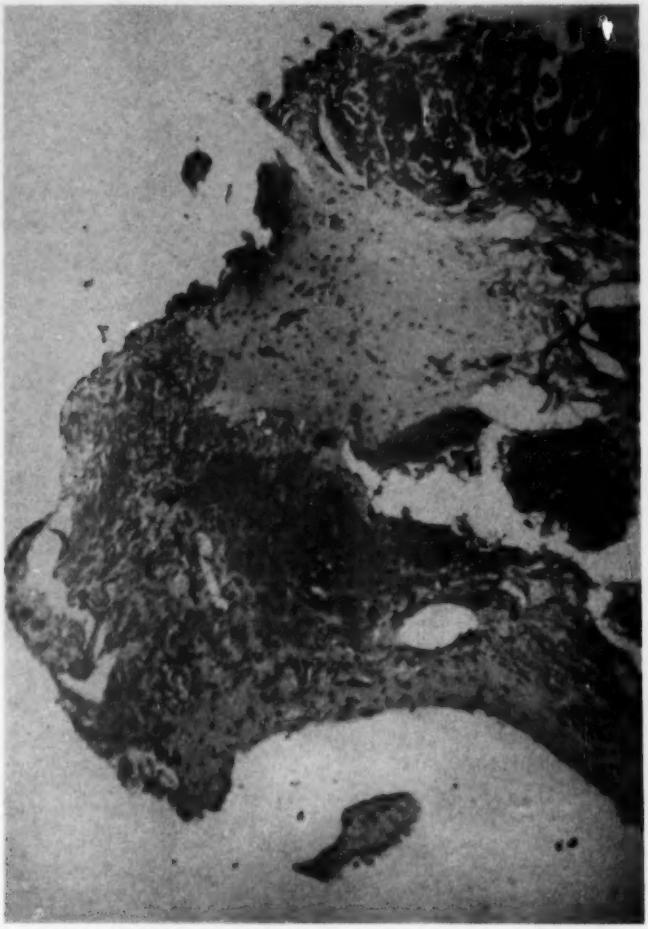

Fig. 3.—Edge of perforation (Case 6). Note diffuse acellular area without evidence of inflammation. The cause of the perforation in this case is unknown. Hematoxylin and eosin; reduced about 10% from mag. × 35.

distention was noted. Owing to respiratory difficulty oxygen therapy was begun. Free air was demonstrated in the abdomen, and a diagnosis of ruptured viscus was made.

The infant was taken to surgery, and a 2 cm. tear along the greater curvature of the stomach was found. Milk curds were present in the abdomen. The defect was sutured, and the milk, evacuated from the abdomen. The child died four hours after surgery.

At the time of autopsy the major findings were within the abdominal cavity. The sutured defect was noted in the stomach and was intact. A diffuse peritonitis was evident. Examination of the other organs, including the brain, was not remarkable.

Microscopically, all sections except those of the stomach and peritoneum were within normal limits. Sections taken through the stomach adjacent to the sutured defect revealed large oval areas of basophilic acellular material (Fig. 4). Inflammatory reaction was minimal. Muscular defects could be demonstrated. Examination of the peritoneum showed the surface to be covered by a thick layer of fibrin, in which were great numbers of gram-negative bacilli and gram-positive cocci. Inflammatory cells were not numerous (Fig. 5).

Comment

In discussing the possible etiologic factors, Greene and Gose [15] state, "It would

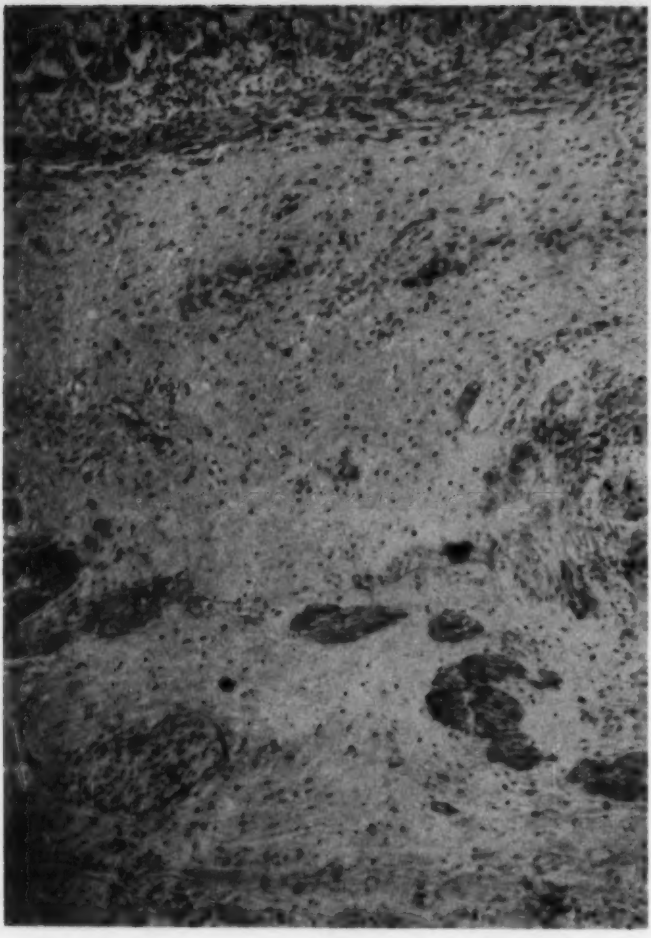

Fig. 4. — Photomicrograph of the wall of the stomach (Case 7), showing a defect in the muscularis. Hematoxylin and eosin; reduced about 10% from mag. × 35.

seem, then, that external pressure of the birth canal, distention of the stomach with or without obstruction, trauma of the mucosa (as by gavage tube) with infection, digestion and perforation and congenital absence or thinning of the muscle play a part. All these factors, however, do not seem to apply to all cases. . . ." Most of the early cases were thought to be due to perforation through an acute peptic ulcer. The ulcer in many of these cases has been attributed to brain injury resulting from a traumatic birth or from cerebral anoxia (so-called "Cushing ulcer"). Kiesewetter [22] stresses the "hypothalamic-pituitary-adrenal mechanism" and the effect of corticosteroids on the stomach as the usual mechanism in producing spontaneous gastric rupture in infants. While brain injury can undoubtedly produce acute peptic ulcers, the majority of the reported cases had no clinical or anatomical evidence to suggest a central nervous system injury, and examination of biopsy or necropsy material from the stomachs tends to rule out acute ulcers in many cases. From the published reports and from personal observation it appears undeniable that acute gastric ulcers can be a cause of spontaneous perforation.[1,4,12,13] Vargas et al.[1] report, "While peptic ulcer

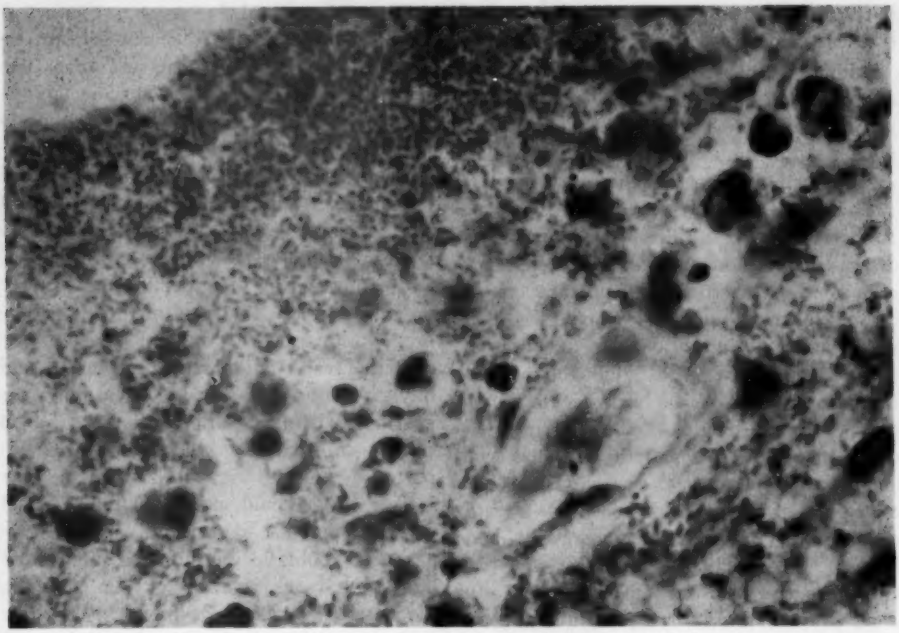

Fig. 5.—Section of the peritoneum from the anterior abdominal wall (Case 7), showing the large numbers of micro-organisms and the relatively mild inflammatory cellular reaction. Hematoxylin and eosin; reduced 15% from mag. × 475.

is almost always the cause of duodenal perforations in infants, it accounts for fewer than one-half of the cases of ruptured stomach." Perforation in our Cases 1 and 3 is thought to be due to gastric ulcers.

Injury due to the introduction of a gastric tube has undoubtedly been responsible for some of the cases of gastric rupture. The majority of reported cases, however, never had such a tube used. Potter[14] stresses that rupture of the stomach due to a gavage tube usually occurs along the greater curvature directly opposite the cardioesophageal opening. Vargas and associates,[1] in illustrating the site of rupture in their Cases 4, 5, and 7, substantiate this statement (Fig. 6). In one case the tube was actually demonstrated to have perforated the stomach wall. It should be stressed that this is not the commonest site of rupture in the reported total-rupture cases. Our Cases 2, 4, and 7 probably can be attributed to such a mechanism, either

Fig. 6.—Diagram of stomach illustrating the sites of perforation (Cases 2 through 7). Stomach in Case 1 ruptured along the posterior wall of the lesser curvature and could not be illustrated on this drawing.

by trauma from a gavage tube or from the tracheal catheter.

Although spontaneous gastric rupture due to septicemia has been reported by several authors,[1,8,14,20] proof of such a cause must be difficult. Durham and Goldstein,[8] in 1934, were apparently the first to report such a case, in a 3-week-old infant with an Escherichia coli septicemia. Hand,[26] in discussing Pseudomonas sepsis, reports a case in which necrosis with perforation occurred in the cecum and emphasizes that gastrointestinal ulceration and perforation can occur. Vargas et al.[1] thought rupture in their Cases 2 and 3 was due to septicemia. In these cases, the incriminated organisms were a hemolytic Streptococcus and a colon aerogenes, respectively. Certainly rupture of the stomach is a rare complication of septicemia, as Weil and Spink,[27] in a study of some 400 cases of Gram-negative sepsis, failed to find any cases of perforated viscus, or indeed, gastrointestinal lesions of any kind. It would seem to me that the presence of a Gram-negative septicemia in cases with a ruptured viscus and peritonitis is not good evidence that the septicemia caused the perforation. It would seem more likely that the sepsis was caused by the perforation. Only if septicemia can be proved to be present before the rupture occurred could such a possible etiology be entertained. Septicemia is not thought to have been responsible for rupture in any of our cases.

Rupture due to congenital muscular defect has been proved beyond reasonable doubt. Brody,[6] in 1940, was the first to report a case of spontaneous rupture in which such a defect existed. Since that time some 20 additional convincing cases have been reported.[1,7,9,11,14,17,20,21,25] While the "proven" cases of congenital muscular defects with rupture account for only slightly more than one-fourth of the reported cases, many other cases may well have been due to such defects, overlooked by the original examiners.[7,20,25] Our Case 7 had such a defect.

The role played by oxygen therapy is difficult to evaluate. A review of the reported cases indicates that less than one-third of the cases in which specific therapy was mentioned received oxygen by any method. Musser,[23] in attributing therapeutic oxygen as the commonest cause of rupture, was apparently unaware of this fact. In those cases receiving oxygen, many received it only after the onset of signs and symptoms of their perforation. In the remaining cases, various methods of administration, such as tracheal catheter, mechanical resuscitator with face mask, and oxygen tent, were used. Exempting the possibility of perforation from a tracheal catheter misplaced in the stomach, as reported by Potter,[14] it is difficult to see how a face mask could cause perforation and nearly impossible to conceive how an oxygen tent could be incriminated. Three of our seven cases received oxygen prior to the onset of signs and symptoms. In Case 4 the stomach may have ruptured because of trauma from an endotracheal catheter.

Birth trauma, thought to be the cause of spontaneous perforation, by some authors,[8,15,19] obviously does not account for more than a very small fraction of the reported cases. Most of these cases are not convincing from the published descriptions. The supposed mechanism acting in these cases is pressure from the birth canal acting on a distended stomach. Rejthar[28] and Millar and associates,[29] in discussing spontaneous rupture of the stomach in adults, stress that the stomach must be "grossly overdistended" from trauma to cause perforation. In addition, most of these perforations, at least in adults, occur along the lesser curvature.[29] Severe gastric distention in a normal infant is rare. Examination of the published reports fails to reveal a history of birth trauma in many instances, a point stressed by Meyer.[25] Postnatal trauma to the chest and abdomen of an infant could conceivably produce gastric rupture, particularly in an infant with acute gastric distention due to a systemic infection.[30] Our Case 5 had a long history of severe

TABLE 2.—*Summary of Cases*

Case	Age	Race	Sex	Premature	Ante-mortem Diagnosis	Perforation		Gavage Tube Prior to Rupture	Septicemia	Oxygen Therapy Prior to Rupture
						Cause	Size & site			
1	9 mo.	N	F	No	Yes	Acute gastric ulcer	1 cm.; posterior wall along lesser curvature just distal to cardioesophageal junction	No	Cultures negative *	No
2	8 day	N	M	Yes (1,650 gm.)	Yes	Trauma	2 cm.; serosal laceration with 5 mm. mucosal defect, fundus, along greater curvature	Yes	No cultures taken *	Yes (incubator)
3	9 day	N	F	Yes (1,070 gm.)	Yes	Acute gastric ulcer	1 cm.; fundus, anterior wall 1 cm. from cardioesophageal junction	No	No cultures	Yes (tracheal catheter)
4	4 day	N	M	Yes (1,000 gm.)	Yes	Trauma	5 mm.; anterior wall	Yes	No cultures *	Yes (tracheal catheter)
5	6 yr.	W	M	No	No	Unknown (massive dilatation)	Large, greater curvature	No	No cultures	No
6	2 wk.	N	M	No	No	Unknown	2.5 cm.; fundus, greater curvature 1 cm. from cardioesophageal junction	No	Cultures negative	No
7	4 day	N	M	Yes	Yes	Trauma & congenital defect	2 cm.; posterior wall of fundus, greater curvature	Yes	No cultures	No

* Patient on prophylactic antibiotics prior to onset of signs and symptoms.

gastric dilatation, which we believe was responsible, through interference with the gastric circulation, for the large area of perforation.

Summary and Conclusions

Rupture of the stomach in children is a rare condition, having been reported less than a hundred times in the literature. In 5,160 autopsies done at the University of Tennessee during the five-year period from August, 1953, to August, 1958, only six examples of gastric rupture in children could be found.

There are multiple causes for gastric rupture, and an attempt to place all cases in only one or two groups is erroneous. Acute gastric ulcers, trauma due to intubation, and congenital muscular defects can all cause rupture independently. There is, in addition, a sizable group for which no etiology has been determined.

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Osteolathyrism

Histopathological Lesions Produced by Semicarbazide and Acetone Semicarbazone

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Osteolathyrism, an experimental disease involving the connective tissues, can be produced in rats by feeding the seeds of certain species of Lathyrus or by feeding certain nitriles, notably aminoacetonitrile or β-aminopropionitrile (BAPN). Pertinent references are given in recent reviews.[1-3] Nonnitriles previously shown to be osteolathyrogenic are β-mercaptoethylamine (Cysteamine) and its corresponding disulfide, Cystamine.[4,5]

The feeding of semicarbazide hydrochloride (SCH) to weanling rats also produces gross skeletal lesions similar to those resulting from the ingestion of BAPN or Lathyrus odoratus (sweet pea).[6] We have recently found that acetone semicarbazone produces similar changes. In an attempt to identify these gross lesions more closely with those produced by BAPN, we have studied the microscopic changes occurring in bones and aortas of rats fed SCH and acetone semicarbazone.

Material and Methods

Weanling, male rats of the Sprague-Dawley strain, weighing 48-60 gm., were used. Experimental diets were prepared by incorporating SCH or acetone semicarbazone into finely ground Rockland Rat Stock Diet. The diets were supplied ad libitum. Animals were killed at intervals, and selected tissues were fixed in 10% buffered formalin. The knee region of a hind extremity from each rat (distal femur—joint proper—proximal tibia and fibula) were decalcified in equal parts of 10% formic acid and 20% sodium citrate. All tissues were dehydrated, cleared, and embedded in paraffin. Six to eight μ sections from eight different levels of the aorta and several parasagittal sections from each knee region were examined. These were stained with hematoxylin and eosin and with a combined elastic and Van Gieson method.

Diets containing SCH were also fed to a few adult rats about 6 months of age. These were examined for gross skeletal lesions and microscopically also for aortic damage.

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Results

The gross lesions produced in weanling rats by diets containing 0.05%-0.25% SCH have been previously described.[6] Acetone semicarbazone, at a dietary level of 0.1%, gave rise to gross skeletal lesions which were indistinguishable from those produced by SCH. At a level of 0.2%, severe depression of growth resulted, with a concomitant delay in the onset of palpably distinguishable exostoses. Prominent exostoses developed also in the adult animals fed 0.25% SCH for 40 days, but primarily at the points of tendon attachment. There was no grossly apparent general thickening of the long bones, such as is seen in growing rats fed these diets.

Histopathologically, the bone and joint lesions in weanling rats of both groups were comparable to those produced by feeding sweet peas or BAPN.[7-9] The periosteum and epiphyseal cartilages were primarily involved. The outer layer of periosteum was affected little, if any. The deep layer showed varying degrees and types of proliferation, especially in regions of tendinous and ligamentous attachments (Fig. 1). The degree of proliferation seemed to vary inversely with the age of

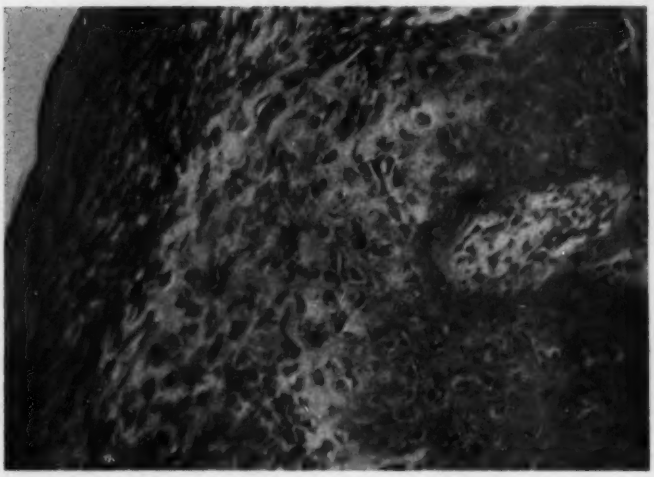

Fig. 1.—Periosteal lesion from weanling rat receiving 0.2% SCH for 28 days, showing fibroblastic proliferation and new bone formation; reduced 40% from mag. × 400.

the animal and directly with the level of the toxic substance in the diet, up to a point which was compatible with life and growth of the animal. The types of proliferation most commonly seen were (1) fibroblastic, with production of collagen; (2) osteoblastic, with production of new bone, and (3) chondroblastic, with production of new cartilage. Basophilic granules or globules were prominent in newly formed bone and cartilage. The latter tended to disappear with increasing age and maturity of the newly formed tissues. No typical "tile-like" or "mosaic" pattern was seen.

The epiphyseal cartilages showed a combination of retrogressive and proliferative changes (Fig. 2). The cartilage cells were generally more abundant than normal and tended to be arranged in groups or clumps, of irregular size and shape, rather than in the usual parallel rows. The cartilage matrix varied from scanty to abundant. The more common retrogressive changes seen in the cells varied from ballooning of cartilage cells to complete destruction and disappearance of the cells. The commonly seen changes in the matrix were (1) acidophilic staining, (2) "fibrillar degeneration," and (3) complete destruction. The destruction of both cells and matrix resulted in spaces or clefts of irregular shape and size. When this was sufficiently extensive, slipping or

Fig. 2.—Epiphyseal lesion from weanling rat receiving 0.2% SCH for 35 days, showing clumping and ballooning of cartilage cells, fibrillar degeneration of matrix, and space or cleft formation; reduced about 40% from mag. × 400.

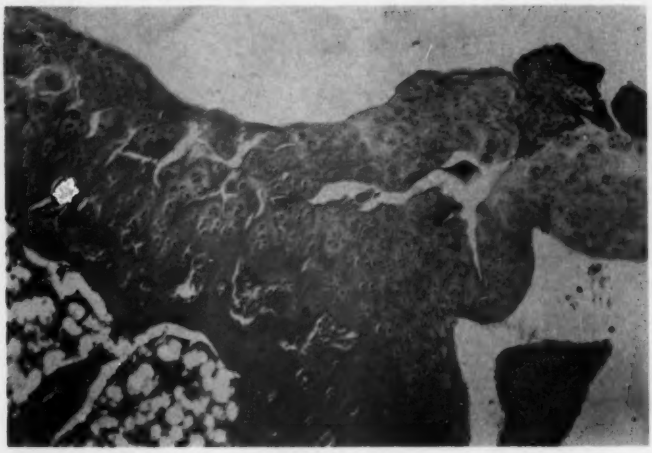

Fig. 3.—Articular lesion from weanling rat receiving 0.1% acetone semicarbazone for 42 days, showing clumping of cartilage cells, space or cleft formation, and proliferation of synovial membrane; reduced about 40% from mag. × 400.

displacement of the epiphyses resulted. The proliferative and destructive changes resulted in great distortion of the skeleton.

In an occasional animal changes occurred in the joint structures proper. The synovial membrane sometimes showed slight proliferation. The articular cartilages occasionally showed changes similar to those seen in the epiphyseal cartilages, proliferation being slight and retrogression predominating (Fig. 3). These changes were not seen in the non-weight-bearing cartilages, i. e., femur-patella. Furthermore, they were seen only in animals with lesions producing marked distortion, such as epiphyseal displacement and/or marked periosteal proliferation. This would seem to indicate that abnormal stress and pressure were factors in the production of these arthritic changes.

Endosteal or osteoclastic destruction and absorption of bone, with thinning of the cortex and enlargement of the marrow spaces, were minimal or absent. The marrow itself was not studied for cytologic detail, but in the routine decalcified specimens the cellularity of the marrow was less in experimental than in control animals (Fig. 4). The difference tended to be more marked in the epiphyses than in the metaphyses. Some fibrosis or sclerosis of the marrow was present but was not prominent, the decreased cellularity being largely

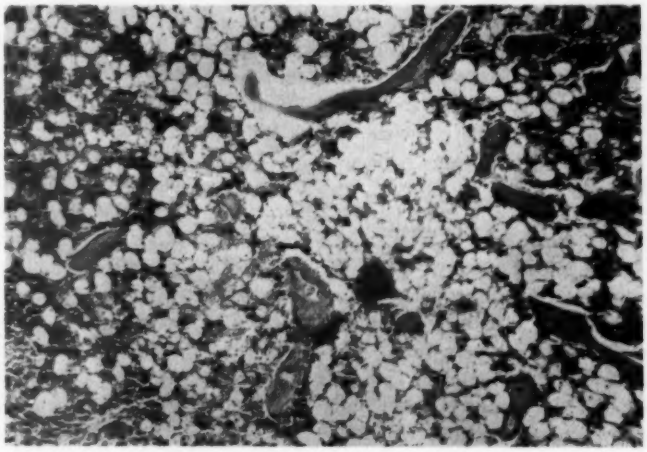

Fig. 4.—Marrow from weanling rat receiving 0.1% acetone semicarbazone for 42 days, showing adipose replacement of hematopoietic marrow. Fibrosis or sclerosis not shown in this section; reduced about 40% from mag. × 400.

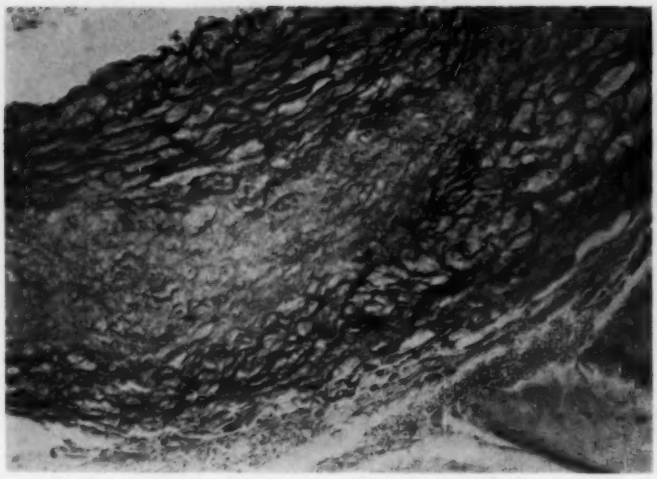

Fig. 5.—Aortic lesion from weanling rat receiving 0.2% SCH for 42 days, showing separation and fragmentation of elastic fibers and early collagen formation; reduced 40% from mag. ×400.

a result of increased adipose tissue. Acute degenerative or necrotic changes of marrow were not seen.

The aortic lesions were not marked in either series of animals. In most animals some of the muscle cells in the media showed a pale swollen cytoplasm, with pyknotic nuclei. The large elastic fibers were slightly separated but otherwise intact. In four animals studied, from the acetone semicarbazone group, these were the only changes seen in the aorta. In a few of the animals of the SCH group, destruction of large elastic fibers in the media was observed (Fig. 5). Repair by collagenous connective tissue was a prominent feature of these lesions. Deposition of calcium was also observed in a number of areas of elastic fiber destruction. The only suggestion of medial dissection or aneurysm formation was the presence of hemosiderin in the media of one semicarbazide group animal.

Comment

The marked similarity of the periosteal, epiphyseal, and articular lesions produced in weanling rats by SCH and acetone semicarbazone to those produced by sweet peas and BAPN [7-9] denotes that this syndrome should tentatively be designated as osteolathyrism.[1] It seems remarkable that such diverse chemicals as BAPN, semicarbazide, and β-mercaptoethylamine [5] should cause apparently identical skeletal lesions. A common mechanism of action appears likely.

Although the aortic lesions were also similar to those seen in animals fed sweet peas or BAPN,[9-13] we did not in this series see aortic lesions comparable in severity to the massive elastic fiber destruction commonly observed in animals fed sweet peas or BAPN. Since most of the animals examined were divided into groups given two different dietary levels of SCH (0.1 and 0.2%) and since animals were killed periodically at time intervals ranging from 7 to 70 days, we can give no accurate estimate of the incidence of elastic fiber destruction in these animals. It appears clear, however, that aortic damage develops more slowly than in rats fed BAPN. The aortic lesions also seem less extensive and less severe than in BAPN-fed animals with comparable skeletal lesions. As with BAPN, the thoracic aorta appears to be more susceptible than the abdominal aorta, although lesions in the abdominal aorta were found. It is noteworthy that, of the two adult animals examined (0.25% SCH for 40 and 61 days, respectively), both showed elastic fiber destruction of the media. Ponseti [7] has reported that rats over

51 days of age showed no aortic damage when fed sweet pea diets.

Summary and Conclusions

The skeletal and aortic lesions produced in weanling rats by feeding semicarbazide hydrochloride or acetone semicarbazone have been examined microscopically. The periosteum showed fibroblastic, osteoblastic, and chondroblastic proliferative changes. The epiphyseal cartilages showed degeneration, necrosis, and atypical proliferation. The marrow showed an increase in adipose connective tissue. Proliferation of the synovial membrane occurred in a few animals. Lesions of the articular cartilages were not common and were predominantly retrogressive rather than proliferative.

No gross aortic lesions were observed. Microscopically, elastic destruction was found in relatively few animals. These areas of destruction exhibited collagenous repair and less frequently dystrophic calcification.

Both skeletal and aortic lesions appear to be similar to those found in experimental lathyrism produced by β-aminopropionitrile (BAPN) or sweet peas. The incidence and severity of the aortic lesions appear to be less than in rats fed sweet peas or BAPN.

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Secondary Lymphoblastomatous Involvement of the Thyroid Gland

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Generally, secondary neoplastic involvement of the thyroid gland is considered uncommon; however, perusal of the relevant literature will show that this is not necessarily so. Several authors have recorded the incidence of grossly visible metastases in thyroid glands of patients dying with malignant neoplasms. Müller,[1] in 1892, found gross metastases in the thyroid gland in 1.5% of 521 patients dying with carcinoma and in 3.1% of patients dying with sarcoma. Thirty years later, Kitain[2] detected metastases in the thyroid gland in 3.1% of cases of carcinoma at necropsy. Wegelin[3] did not consider metastases in the thyroid gland an exceptional rarity and mentioned that he had encountered them 34 times between 1900 and 1922 in necropsies performed at the University of Bern. The total number of necropsies was not mentioned. In 170 consecutive necropsies on persons who died with malignant tumors, Willis[4] found metastases in the thyroid gland in 5.2%. He pointed out that casual bilateral sectioning of the gland is not enough to reveal this percentage of metastases. It was only by careful routine sectioning that it was possible for him to find such a high incidence.

All of the above publications dealt with only grossly visible metastases to the thyroid gland. Rice,[5] in 1934, carried the study further in searching for microscopic as well as macroscopic metastases. In this way, he was able to find metastases, 5 grossly and 4 microscopically visible, in a series of 89 necropsies on persons dying with malignant disease. The 10% involvement in this series was scarcely approached in the recent study of Mortensen et al.[6] They performed minute gross and microscopic examination of thyroid glands removed from 467 patients at necropsy, all of whom were known to have, or have had, a histologically proven malignant neoplasm. Their method of examining the thyroid glands appeared to have been more painstaking than those of previous authors, but even so, the incidence of secondary involvement of the thyroid gland was only 3.9%. The neoplasms in their series with the highest incidence of involvement of the thyroid gland were those of lymphatic tissue. Five of the thirty-seven tumors of lymphatic tissue involved thyroid gland, an incidence of 14%.

Considering that the thyroid gland has a fundamental relation to lymphatic tissue[7,8] and may normally contain lymphocytes,[9] it is not surprising that secondary involvement by lymphoid tumors was so high in Mortensen's[6] series. With a view to ascertaining the frequency of secondary involvement of the thyroid gland by tumors of lymphoid tissues ("lymphoblastomas"), an investigation was carried out on thyroid glands obtained at necropsy from 300 cases of generalized lymphoblastoma. In addition to determining this frequency, an attempt was made to ascertain the manner in which the thyroid glands were involved.

Material and Method

Under the term "lymphoblastoma" are included several related but pathologically distinct entities, namely, the leukemias—myelogenous, lymphocytic, monocytic, and stem cell—and the nonleukemic lymphoblastomas—giant follicular lymphoblastoma,

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Hodgkin's disease, lymphosarcoma, reticuloendothelial-cell sarcoma, and multiple myelomatosis.

The material used in this investigation was collected from 300 necropsies on the bodies of patients who died with generalized lymphoblastoma in the University of Michigan Hospital. At least two sections were studied from each thyroid gland, and these usually were from each lateral lobe. When the microscopic findings were unsatisfactory, more sections were cut from the stored gross necropsy material. Routine hematoxylin and eosin staining methods were used.

The degree of lymphoblastomatous involvement of the thyroid gland was assigned to one of three grades. Grade 1 indicated minimal, although definite, involvement; Grade 3 denoted large, easily identified sheets of neoplasm, areas that could not be viewed completely in a single low-power field of the microscope (an area 1.3 mm. in diameter on the section); while Grade 2 infiltrations were those which fell between Grades 1 and 3.

In all examples of Grade 3 involvement, the stored gross material was carefully reexamined in an attempt to identify macroscopic foci which might have been overlooked originally. Furthermore, any large masses of thyroid gland were sectioned more thoroughly. In this material, several gross lesions were discovered, and their lymphoblastomatous nature was confirmed by microscopic examination.

Differentiating non-neoplastic lymphocytic aggregations from lymphocytic leukemic infiltrations often was difficult, and the criteria adopted to differentiate these conditions depended upon two factors, namely, the maturity of the cells and the extent of infiltration. Infiltrations of immature lymphocytes were considered to be evidence of leukemic involvement, as were infiltrations of lymphocytes, whether mature or immature, which extended in one direction at least one low-power field and did not show germinal centers. This last qualification was important because a thyroid gland in which there was lymphoid hyperplasia might show such large infiltrations of mature lymphocytes, but it was rare that germinal centers were not found somewhere in the section. Even when applying these criteria, it was occasionally impossible to decide whether the infiltrations were neoplastic or not; consequently, several of such equivocal infiltrations had to be regarded as non-neoplastic.

Findings

Of the 300 necropsies studied with generalized lymphoblastoma, 53 (17.7%) showed involvement of the thyroid gland. There were 188 examples of leukemic lymphoblastoma, and 34 (18.2%) of these showed involvement of the thyroid gland. Thyroid glands from the 112 patients with nonleukemic lymphoblastoma were involved in 19 instances (16.9%).

Gross involvement of the thyroid gland by lymphoblastoma was encountered 11 times: in one case of myelogenous leukemia, in one of monocytic leukemia, in four cases of reticuloendothelial-cell sarcoma, and in five of lymphosarcoma.

The entity with the highest incidence of thyroid gland involvement was reticuloendothelial-cell sarcoma (28.6%), followed by lymphosarcoma (26.3%) and lymphocytic leukemia (23.5%). Probably, in reality,

TABLE 1.—*Thyroid Glands from Necropsies*

	Glands				
	Examined,	Involved			
Lymphoblastoma	No.	No.	%	Women, %	Men, %
Myelogenous leukemia	85	16	18.8	18.6	19.0
Lymphocytic leukemia	51	12	23.5	33.3	18.0
Monocytic leukemia	29	4	13.8	18.2	11.1
Stem-cell leukemia	23	2	8.7	25.0	6.2
Total leukemic lymphoblastomas	188	34	18.2	22.4	15.2
Reticuloendothelial-cell sarcoma	21	6	28.6	22.2	33.3
Hodgkin's disease	41	3	7.3	—	14.3
Lymphosarcoma	38	10	26.3	47.1	9.5
Multiple myelomatosis	12	..	....	....	....
Total nonleukemic lymphoblastomas	112	19	16.9	20.5	14.3
Grand total	300	53	17.7	21.6	15.4

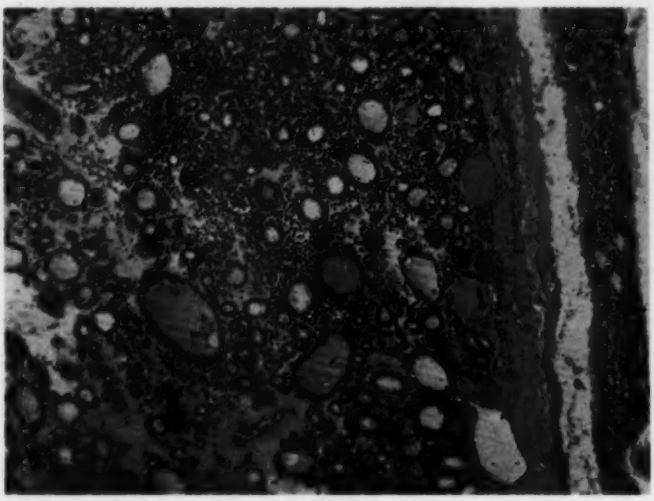

Fig. 1. — Myelogenous leukemic cells involving an adenoma of the thyroid gland in which there are retrogressive changes. The capsule of the adenoma is at the right. Hematoxylin and eosin stain; reduced 15% from mag. × 80.

the last figure would be higher were it not for the stringent criteria used to decide whether a lymphocytic infiltration was leukemic or not. A statistical representation of the secondary involvement of the thyroid gland by lymphoblastomas is presented in Table 1.

In one case of myelogenous leukemia (Fig. 1), one of lymphatic leukemia, and three of lymphosarcoma (Fig. 2) the lymphoblastomatous involvement appeared to be confined entirely to an adenoma in each gland. In spite of this, adenomatous thyroid glands were not found to be seats of secondary lymphoblastomatous involvement more often than nonadenomatous glands. Nor was there found to be any essential difference in weight between those thyroid glands which were and those which were not involved by lymphoblastoma. There were many types of pathologic change in the thyroid glands which were

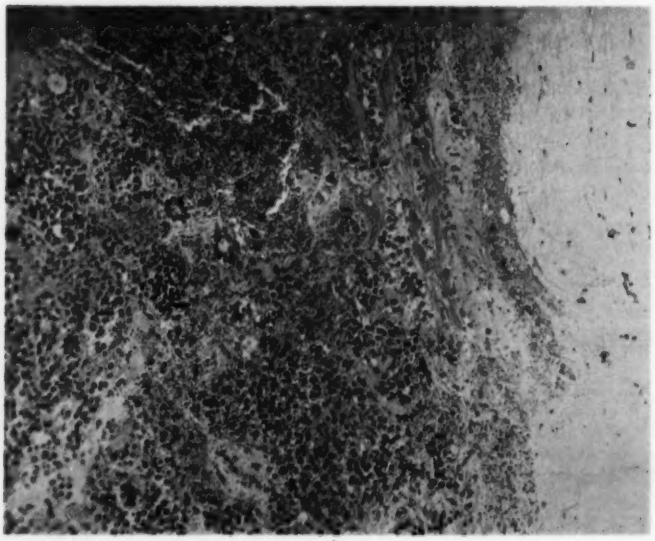

Fig. 2. — Lymphosarcoma cells within a thyroid adenoma in which there are retrogressive changes. Thick hyalinized connective tissue capsule on the right. Hematoxylin and eosin stain; reduced 15% from mag. × 140.

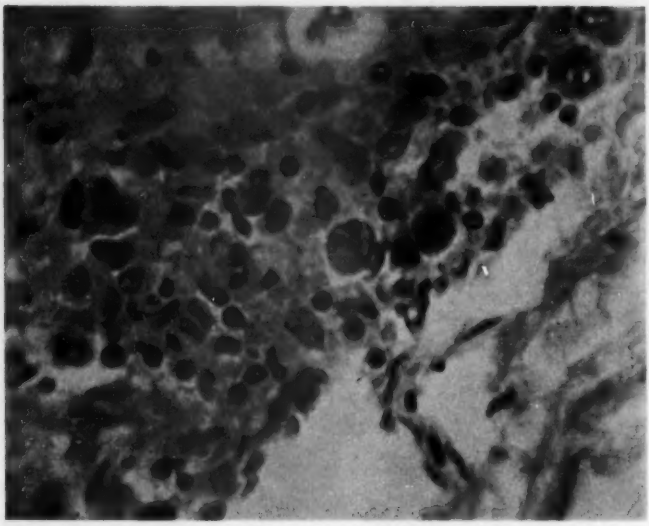

Fig. 3.— Reticuloendothelial-cell sarcoma involving the thyroid gland. Small focus of lymphoblastomatous cells in the stroma of the gland. In addition to the obvious neoplastic cells, some of which show mitoses, there is hypertrophy of neighboring reticuloendothelial cells, which appear to be in stages of transition into neoplastic cells. Hematoxylin and eosin stain; reduced 10% from mag. × 630.

involved by the lymphoblastomas. Most of these changes, however, were independent of the lymphoblastomatous involvement; they did not occur more frequently in those thyroid glands which showed involvement than in those which did not. It is interesting to note that there was a distinctly higher incidence of involvement of the thyroid gland in women than in men (Table 1).

In general, involvement by the nonleukemic lymphoblastomas was minute, 76.5% being less than Grade 3; while involvement by the nonleukemic lymphoblastomas was more extensive, only 20.1% being less than Grade 3. Also, most leukemic foci were situated solely at the periphery of the gland, whereas the nonleukemic infiltrations usually involved both periphery and interior.

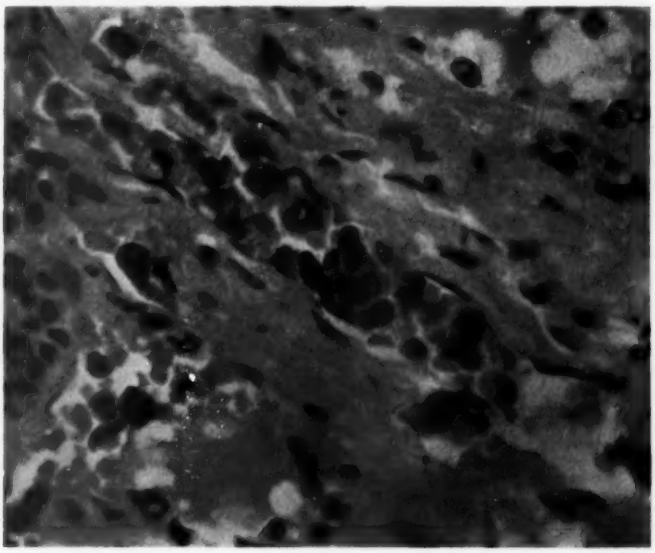

Fig. 4. — Reticuloendothelial-cell sarcoma involving the thyroid gland. Neoplastic cells in capillaries. Hematoxylin and eosin stain; reduced about 10% from mag. × 630.

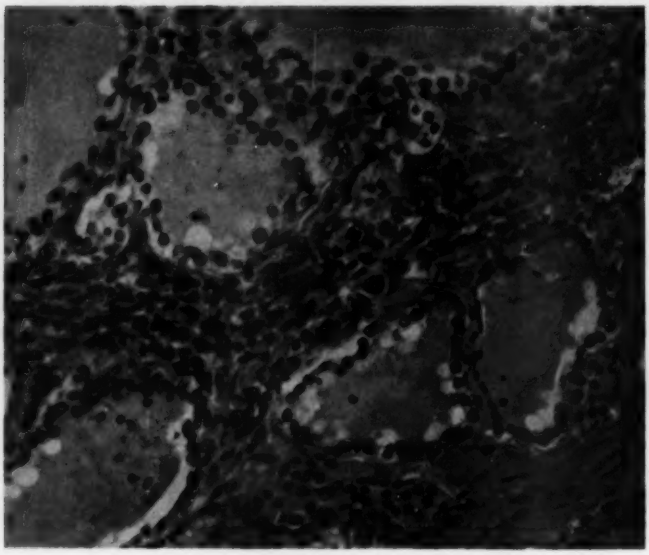

Fig. 5.—Hodgkin's disease involving the thyroid gland. Hyperplasia, hypertrophy, and hyperchromatism of the pericapillary, interfollicular reticuloendothelial cells. Hematoxylin and eosin stain; reduced about 10% from mag. × 330.

In one example of reticuloendothelial-cell sarcoma and one of Hodgkin's disease it appeared that incipient foci of neoplasm were developing through neoplastic transformation of reticuloendothelial type cells within the substance of the glands (Figs. 3, 4, and 5).

Involvement of the thyroid gland by contiguity of cervical lymph nodes did not appear to have occurred in any case. However, it was found that involvement of the thyroid gland took place more frequently when the cervical lymph nodes were involved in the neoplastic process (Table 2), this probably being due to a wider dissemination of the disease.

The lymphoblastomatous involvement of the thyroid gland was evident clinically in two cases of lymphosarcoma, but in neither was enlargement of the thyroid gland an initial or distressing symptom.

TABLE 2.—*Lymphoblastomatous Involvement of Thyroid Gland and Cervical Lymph Nodes*

	Lymphoblastoma, %		
	Leukemic	Nonleukemic	Grand Total, %
With involvement of cervical lymph nodes	82.3	68.4	77.4
Without involvement of cervical lymph nodes	10.3	16.7	12.8

Comment

In the present study, leukemic infiltration was found in 18% of thyroid glands of patients who died of leukemia; this is a higher percentage of secondary neoplastic involvement than that recorded in other reported studies. That it should be so high is not surprising, since the thyroid gland, second only to the adrenal glands, is, by weight, the most highly arterialized organ in the body.[4] Zeidman et al.[10] demonstrated that the number of metastases developing in the lungs of mice, after the intravenous injection of suspensions of different numbers of transplantable sarcoma cells, was proportionate to the number of living embolic cells injected. Consequently, the rich blood supply would render the thyroid gland particularly susceptible to metastatic involvement in leukemias.

In this study, it was found that in the cases of leukemia the neoplastic involvement was predominantly confined to the capsular and subcapsular regions of the thyroid gland, and the areas of involvement usually were minute. Two reasons can be

advanced for this: (1) When the arteries enter the thyroid gland, they divide into small branches which offer mechanical resistance to the progress of the neoplastic cells which then leave the vessels, or (2) the peripheral and intra-adenomatous distribution may be explained by the "soil" hypothesis.

Using Paget's [11] simile of "seeds" falling on different "soils," it may be that the "soil" surrounding the thyroid follicles is unsuitable for the establishment of the leukemic infiltrations. The occurrence of lymphoblastoma cells solely within adenomas of the thyroid gland requires an explanation: Perhaps adenomatous changes in the thyroid tissue favor growth of metastases. Willis [12] thought that it was improbable that altered vascular conditions in pathologic thyroid tissue favored the arrest of tumor emboli for two reasons: (1) Diminished blood supply of altered areas of tissue must reduce the number of emboli, and (2) neoplastic emboli were seldom so minute that slight differences in caliber of capillaries would not influence the incidence of metastases in different tissues. He noted that the thyroid gland is richly oxygenated and poor in carbohydrate—two factors which may be significant, since the growth of certain tumors is favored by anaerobic conditions.[13] Perhaps the peculiar chemical character of the thyroid parenchyma plays a part in rendering this tissue an inhospitable "soil" for embolic cells. However, the morphological observations made in this study on the distribution and quantity of the leukemic infiltrations may be evidence in favor of the "soil" hypothesis.

In most of the examples of nonleukemic lymphoblastoma, the infiltrations were extensive (79% were Grade 3) and involved much of the thyroid gland. An explanation for this may be found if the courses of the two groups of patients, the leukemic and the nonleukemic, are compared. This period was the interval between the onset of symptoms which could be attributed to the lymphoblastomatous disease and the time that the patient died. The average duration of the disease in the leukemic patient was 0.8 year, while that in the nonleukemic was 2.7 years. The longer period in the cases of the nonleukemic lymphoblastoma would permit the lymphoblastomatous process to become established peripherally and spread inward.

It is probable from the observations made in this study that lymphoblastomas most frequently involve the thyroid gland by way of the blood stream. Neoplastic transformation of the normal reticuloendothelial-cell component of the thyroid gland occurs, though much less frequently, and invasion by contiguity is rare.

The fact that none of these patients experienced enlargement of the thyroid gland during life as a primary manifestation of the lymphoblastomatous process does lend evidence, though indirectly, to the suggestion of Welch et al.[14] that primary malignant lymphoma of the thyroid gland is usually so characteristic in its clinical manifestations as to constitute a syndrome.

Summary

The frequency of involvement of the thyroid gland in 300 necropsies on bodies of patients with generalized lymphoblastomas was determined. Over-all, 17.7% of the thyroid glands showed lymphoblastomatous involvement, 18.2% in the leukemic and 16.9% in the nonleukemic lymphoblastomas.

Particular attention was directed to the actual positions and sizes of the lymphoblastomatous infiltrations. Their small size and peripheral or solely intra-adenomatous distribution may be determined by the thyroid gland's being poor "soil" for exogenous neoplasms.

Involvement of the thyroid gland by lymphoblastomas apparently occurs most frequently by way of the blood stream.

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Congenital Tricuspid Atresia

Report of Two Cases

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Tricuspid atresia is one of the rarest congenital cardiac diseases. Recently we had experience with one such case, and, on reviewing our autopsy files, another one was discovered. Fifty cases with congenital cardiac disease have been autopsied at the Huron Road Hospital, Cleveland, during the period Jan. 1, 1937, to Sept. 1, 1958. Thus the incidence of tricuspid atresia in this series is 4% of congenital heart diseases (Table).

Incidence of Major Congenital Cardiac Lesions in Fifty Autopsied Cases of Congenital Heart Disease at Huron Road Hospital, Cleveland

Lesion	No.
Patent ductus arteriosus	8
Ventricular septal defect	8
Truncus arteriosus with ventricular septal defect	7
Transposition of the great vessels	6
Aortic stenosis	4
Coarctation of the aorta	3
Pulmonary stenosis with closed ventricular septum	2
Cor biloculare	2
Pulmonary stenosis with open ventricular septum	2
Mitral and aortic atresia	1
Mitral stenosis	1
Aortic atresia	1
Tricuspid stenosis	1
Tricuspid atresia with transposition of the great vessels	1
Tricuspid atresia with truncus arteriosus	1
Miscellaneous	2
Total	50

Report of Cases

CASE 1.—A boy was born on Aug. 7, 1954, in our hospital. Pregnancy and delivery were normal. He was markedly cyanotic at birth. However, cyanosis gradually subsided but did not disappear entirely. Cyanosis always increased with crying and exertion. Subsequently, he was hospitalized on five separate occasions—three times for treatment of upper respiratory infections and pneumonia and twice for evaluation of his cardiac status. There was history of cardiac decompensation on several occasions, for which he was given digitalis and mercurial diuretics. On Aug. 10, 1958, at the age of 4 years, he was hospitalized for the sixth time, for cardiac surgery.

Physical examination revealed a well-developed and well-nourished white boy, not in acute distress. There was a slight cyanosis of the mucous membranes. There were no signs of venous congestion. The neck veins were not distended, and the liver was not palpable. The lungs were clear. A Grade 3 harsh, blowing systolic murmur was audible over the entire precordium, loudest at the lower left sternal border. There was no evidence of clubbing of the fingers.

The hemoglobin was 14.4 gm. per 100 ml. of blood. The hematocrit was 43%.

Several electrocardiograms were taken, and all revealed left ventricular preponderance, with left axis deviation and high notched P-waves.

Radiological examination of the heart showed a globular appearance, with marked left ventricular enlargement and absence of right ventricular shadow. The lung fields showed increased pulmonary vascularity. On successive studies there was a progressive increase in size of cardiac shadow.

Cardiac catheterization was performed when the patient was 7 months old. The catheter could not be passed into the right ventricle but entered with ease the left atrium and left ventricle. The data were consistent with tricuspid atresia. A second cardiac catheterization was performed seven months prior to operation. The results this time were equivocal, and the final report was intra-auricular and intraventricular defect. At this time, angiocardiography was also attempted unsuccessfully.

A provisional diagnosis of tricuspid atresia was made. However, the exact status of the cardiac anomalies could not be evaluated. There was also a strong suspicion of atrioventricularis communis.

On Aug. 12, 1958, the patient was subjected to an exploratory thoracotomy. With the findings, operative interference being not indicated, the chest was closed. However, the patient did not recover from the anesthesia and died within 18 hours.

Postmortem Findings

The body was fairly well developed and well

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nourished. There was a slight cyanosis of the lips. There was no clubbing of the fingers or toes.

Abdomen: No abnormality was noted. The liver was not enlarged and the abdominal organs were normally related.

Thorax:

The lungs were voluminous. The right lung had two lobes, the left lung, three lobes. The mediastinum was wider than normal because of the cardiac enlargement, the apex being in the fifth intercostal space at the left anterior axillary line.

The points of chief interest were in the heart and the great vessels. The heart weighed 150 gm. The pulmonary trunk arose dorsal and to the left of the aorta. The branches of the aortic arch—the innominate, the left common carotid, and the left subclavian—were given off normally. The aorta had a circumference of 3 cm. throughout except at a point just beyond the origin of the left subclavian artery, where the circumference of the aorta was 7 mm. Immediately distal to this coarctation there was a large patent ductus arteriosus, communicating with the main trunk of the pulmonary artery.

Examination of the interior of the right atrium showed that the superior and the inferior venae cavae and the coronary sinus entered this chamber normally. There was tricuspid valve atresia, and no tissue was recognizable as that of the tricuspid valve. The only outlet of the right atrium was a patency of the atrial septum, represented by a retention of the fetal type of foramen ovale with a fully developed valve of the foramen ovale. The right atrial chamber and more particularly the left atrial chamber were wider than normal. The left atrium received the pulmonary veins and communicated with the right atrium through the patent foramen ovale and also with the left ventricle through a normally formed and wide mitral valve, which measured 10.5 cm. at its circumference. The left ventricle was large and hypertrophied, measuring 14 mm. at its greatest thickness. The pulmonary trunk was considerably wider, measuring 6 cm. at its circumference, and it arose from the left ventricle. The pulmonary valve had three well-formed cusps. The right ventricle was rudimentary and appeared as a simple outpouching of the large left ventricle along its right upper aspect. It communicated freely with the left ventricle through a large ventricular septal defect. The transposed aorta arose from this small right ventricle. The aortic valve, which measured 3 cm. at its circumference, had three well-formed cusps. The coronary arteries arose from the aorta in their usual position. (Fig. 1).

Histology

Sections from the heart revealed acute pericarditis (probably operative). Sections from the middle lobe of the left lung revealed massive hemorrhage and beginning acute bronchopneumonia. Sections from the rest of the lung showed marked congestion and some sclerosis of the pulmonary arteries. The liver, the spleen, and the kidneys showed marked congestion.

Diagnosis

1. Congenital heart disease: (*a*) tricuspid atresia, (*b*) transposition of the great vessels, (*c*) dilatation of the pulmonary artery, (*d*) patent foramen ovale, (*e*) interventricular septal defect, (*f*) coarctation of the aorta, (*g*) patent ductus arteriosus, and (*h*) hypoplasia of the right ventricle.
2. Trilobe left lung and bilobe right lung.
3. Pulmonary arteriosclerosis (slight).
4. Pulmonary hemorrhage.
5. Acute and passive congestion of the viscera.
6. Acute pericarditis (probably operative).

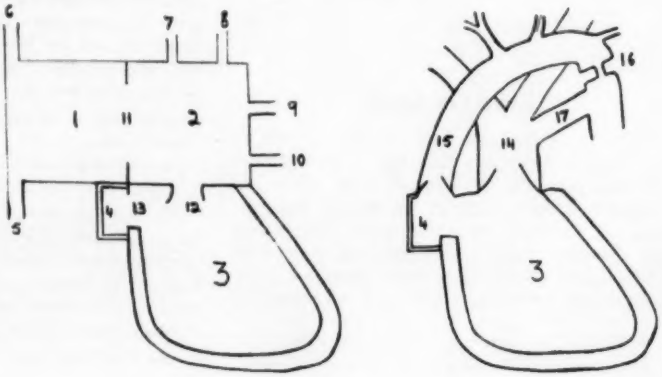

Fig. 1 (Case 1).—Diagram of circulation: (*1*) right atrium, (*2*) left atrium, (*3*) left ventricle, (*4*) right ventricle, (*5*) inferior vena cava, (*6*) superior vena cava, (*7, 8, 9,* and *10*) pulmonary veins, (*11*) patent foramen ovale, (*12*) mitral valve, (*13*) interventricular septal defect, (*14*) dilated pulmonary artery, (*15*) aorta, (*16*) coarctation of aorta, and (*17*) patent ductus arteriosus.

CASE 2.—A boy was born on Oct. 19, 1949. Pregnancy and delivery were uneventful. The baby cried at once after delivery but was noticed to be cyanotic. Except for persistent cyanosis, physical examination of the baby was completely negative. The heart sounds were normal, and there was no murmur. His hemoglobin was 110%, and the red blood cell count was 5,460,000 per cubic millimeter.

Electrocardiography revealed sinus tachycardia, with a rate of 158 per minute, low inverted T's in Leads I and II, tall peaked P's in Leads II and III, and axis normal.

Radiological examination of the chest was negative except for thymic enlargement with tracheal compression. The patient was given 100 r of x-ray to the thymus, with no improvement. A cardiologist was consulted, but before further studies could be performed the baby died, on the ninth day after delivery.

Postmortem Findings

The body was well developed and well nourished. He showed a moderate degree of cyanosis.

Abdomen: No abnormality was noted. The liver and the spleen were of average size, and the abdominal organs were normally related.

Thorax: The lungs were well expanded. There were numerous small areas of hemorrhage on the surface of the lungs. The heart was of average size for a newborn. There was one vessel leaving the ventricular part of the heart and having a diameter of 9 mm. This formed a regular aortic arch from which arose the innominate, the left common carotid, and the left subclavian. The pulmonary trunk, the right and left pulmonary arteries, and the ductus arteriosus were absent. The lungs were supplied solely by two bronchial arteries, each arising separately from the arch of the aorta at the level of the origin of the left common carotid.

Examination of the interior of the right atrium showed that the superior and the inferior venae cavae and the coronary sinus entered this chamber normally. There was complete tricuspid atresia. The foramen ovale was patent, and through it the right atrium communicated with the left atrium. The left atrium received the pulmonary veins and through a normally developed bicuspid mitral valve communicated with the ventricular portion of the heart, which revealed only a single cavity, the interventricular septum being completely absent. There was a single arterial trunk which arose from this single ventricle. The valve of this trunk was formed of three cusps. The two coronary arteries arose from this main arterial trunk (Fig. 2).

Histology

Except for considerable congestion of the viscera and a few small areas of hemorrhage and atelectasis in the lungs, no abnormality was found on sections of different organs.

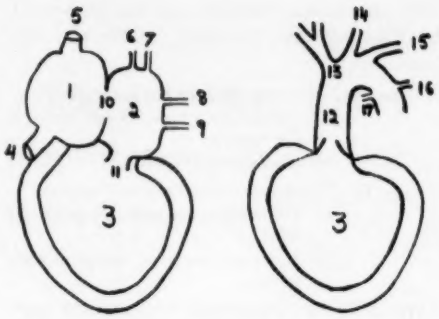

Fig. 2 (Case 2).—Diagram of circulation: (*1*) right atrium, (*2*) left atrium, (*3*) single ventricle, (*4*) inferior vena cava, (*5*) superior vena cava, (*6, 7, 8,* and *9*) pulmonary veins, (*10*) patent foramen ovale, (*11*) mitral valve, (*12*) persistent truncus arteriosus, (*13*) innominate artery, (*14*) left common carotid artery, (*15*) left subclavian artery, and (*16* and *17*) bronchial arteries.

Diagnosis

1. Congenital heart disease: (a) tricuspid atresia, (b) patent foramen ovale, (c) single ventricle, (d) persistent truncus arteriosus, and (e) hypoplasia of pulmonary arteries and ductus arteriosus.
2. Generalized acute passive congestion.
3. Focal areas of atelectasis, hemorrhage, and infarction of lungs.

Comment

Keith, Rowe, and Vlad (1958),[2] reviewing the literature, found 66 instances of congenital tricuspid atresia in 2,127 postmortem cases of congenital heart disease (incidence of 3.1%).

In all cases of tricuspid atresia the heart functions as a two-chambered heart. These hearts have certain anatomic features in common, although some differences may exist from case to case. Common to all the cases are (1) atresia of the tricuspid orifice, (2) patency of the atrial septum, and (3) a large mitral orifice leading into a large ventricular chamber. A classification depending upon differences between specimens was first attempted by Kühne (1906),[3] who distinguished between two groups (with and without transposition of the great vessels).

This anatomical classification was elaborated by Edwards and Burchell (1949),[1] as follows:

Type I. No transposition of the great vessels
- (*a*) Pulmonary atresia, closed ventricular septum
- (*b*) subpulmonary stenosis

Type II. Transposition of the great vessels
- (*a*) Pulmonary or subpulmonary stenosis
- (*b*) No pulmonary or subpulmonary stenosis

Type II(b) is illustrated by our first case. The second case is an oddity, as the association of tricuspid atresia with persistent truncus arteriosus, to our knowledge, has never been previously reported.

Summary

Two cases of congenital tricuspid atresia are presented.

A review is made of the incidence of major congenital cardiac lesions in 50 autopsied cases of congenital heart disease.

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An Infarct-like Myocardial Lesion and Other Toxic Manifestations Produced by Isoproterenol in the Rat

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Progress in the study of myocardial necrosis has been handicapped by the lack of a simple and reliable method for the production of this lesion in experimental animals. The studies of investigators working to this end for nearly a hundred years have been reviewed recently by Taylor et al.[1] Surgical methods of producing coronary infarcts in the dog were disappointing because of the difficulty in achieving standardized lesions.[2,3] More recent improvements in technique[4-6] have resulted in greater uniformity in the lesions, but the methods are still expensive, time-consuming, and coupled with a high mortality. Although the rat is often more readily available than the dog, only a few authors have attempted to produce surgical lesions in this species.[7-9]

Many attempts were made in Germany, particularly during the late 30's, to produce the morphological equivalent in experimental animals of human angina pectoris. Much of this work centered in the Institute of Büchner.[10] These workers found that hemorrhagic shock,[11,12] orthostatic collapse,[13-15] or low atmospheric pressure[16,17] could produce disseminated cardiac necrosis in the rabbit. The lesions in these animals were small foci of necrosis and inflammatory reaction, particularly prominent in the subendocardial areas of the left ventricle and the papillary muscle. The mechanism by which the lesions were produced under these circumstances was nonspecific, since in other experiments similar lesions were described in this species after only forced restraint[18] or the administration of sodium chloride.[14] According to this school carbon monoxide poisoning,[18,19] anaphylactic shock,[14] insulin shock,[20] histamine shock,[13, 14,18,21] or toxic doses of pentylenetetrazol (pentamethylenetetrazol)[20] would also produce disseminated myocardial necrosis in the rabbit.

Other authors, working with the cat, were able to produce similar lesions in the heart after large doses of epinephrine,[18] vasopressin (Pitressin),[22] or digitalis.[23] Banting and his group were successful in producing myocardial necrosis in the dog and rat by the administration of large doses of acetylcholine[24,25] or by vagal stimulation of unanesthetized or lightly anesthetized dogs.[26] Horswell was unable to produce these lesions in the dog with acetylcholine.[27] Robertson et al.[28] produced a striking focal myocarditis with necrosis by the intravenous injection of papain to rabbits, rats, or mice.

Myocardial necroses related to coronary arterial lesions have been reported after corticotropin (ACTH) administration,[29] with adrenal-regeneration hypertension,[30] with renal hypertension,[31] or after coronary atherosclerosis produced in rats by dietary means.[32,33]

A new approach to the study of myocardial necrosis has been described by Selye.[34] Rats sensitized by the oral administration of monosodium phosphate developed myocardial necrosis following the injection of 2α-methyl-9α-chlorocortisol. Lesions were found in many areas of the heart in these animals but were most frequently located in the wall of the right ventricle and papillary muscles.

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From Research Laboratories, Ayerst, McKenna & Harrison, Ltd.

In a recent preliminary publication [35] we have described the production of myocardial necrosis in the rat by a wide range of doses of isoproterenol (1-[3′,4′,-dihydroxyphenol]-2-isopropylaminoethanol) hydrochloride (DIH). This paper describes in detail the gross and microscopic lesions in these animals. It is evident from these data that careful choice of dosage of this compound allows the production of standardized myocardial lesions of predictable severity.

Material and Methods

The animals used in this study were male Wistar rats weighing an average of 258 ±10 gm. and females weighing 200 ±4 gm. They were housed under controlled conditions of temperature and humidity and fed a commercial rat food. As a basis for the choice of doses used in subsequent tests an acute toxicity test was performed to determine the median lethal dose (L. D_{50}). DIH was administered subcutaneously in aqueous solution at four dose levels to groups of 10 animals. The mortality rate present in the groups at 24 hours was recorded and used to calculate the L. D_{50} by the method of Litchfield and Wilcoxon.[36] In the remaining experiments, starting with the L. D_{50}, 16 gradually decreasing doses were tested, each dose being one-half of the previous one. The injections were given subcutaneously to five male and five female rats on two consecutive days; control rats received distilled water. Forty-eight hours after the first injection the rats were killed and autopsied. The hearts were removed and weighed, and the gross lesions were graded according to the following system: Grade 0: no lesions; Grade 1: mottling of the apex and distal parts of the left ventricle caused by intermingled pale and dark red streaks; Grade 2: well-demarcated necrotic areas limited to the apex; Grade 3: large infarct-like necrosis involving at least one-third of the left ventricle and extending to adjacent areas of the interventricular septum and right ventricle; Grade 4: large infarct-like necrosis involving more than half of the left ventricle interventricular septum and extending to the distal portion of the right ventricle.

After fixation in Bouin's fluid frontal sections of the heart, which included both auricles, ventricles, and interventricular septum, were embedded in paraffin. Sections cut at 5μ were stained with hematoxylin and eosin, Cason's trichrome,[37] and periodic acid Schiff (P. A. S.).[38] Frozen sections of the heart were stained with Sudan IV. Microscopically the hearts were graded as follows: Grade 0: no lesions; Grade 1: focal lesions of the subendocardial portion of the apex and/or the papillary muscle, composed of fibroblastic swelling or proliferation and accumulation of histiocytes; Grade 2: focal lesions extending over wider areas of the left ventricle, with right ventricular involvement (lesions included also edema, mottled staining, fragmentation, and segmentation of muscle fibers); Grade 3: confluent lesions of the apex and papillary muscles, with focal lesions involving other areas of the ventricles and the auricles (The lesions included vacuolar and fatty degeneration, granular disintegration, and hyaline necrosis of the muscle fibers. Marked capillary dilatation was present with hemorrhages. Extensive edema, occasionally with a mucoid component, caused sequestration of muscle fibers.); Grade 4: confluent lesions throughout the heart, including infarct-like massive necrosis, with occasionally acute aneurysm or mural thrombi. (The latter lesions were usually apical but also occurred in the papillary muscles or right ventricle. The lesions were similar in character to those in Grade 3.) Histological sections were also made of other organs in which gross lesions were observed, and in two additional animals from each group sections were made of the brain, pituitary, lung, liver, kidney, adrenal, and pancreas.

Results

The L. D_{50} of DIH after subcutaneous administration to the rat was found to be 680 mg. per kilogram. When this dose was administered to a group of rats on two consecutive days the mortality was 80%. Doses of 340, 170, and 85 mg. per kilogram caused deaths of 90%, 50%, and 10% of the animals, respectively, during the experimental period. Sporadic deaths occurred at lower doses: one at 21.2 mg. and two at 1.3 mg. per kilogram. These deaths occurred in animals with particularly severe heart lesions. The animals which died at doses of 170 mg. per kilogram or greater also revealed liver and kidney necrosis, hydrothorax, and hemorrhagic lung edema.

The animals which received doses of 5.25 mg. per kilogram or greater showed characteristic symptoms after injection. Within 20 minutes of treatment, the animals assumed unusual postures. Some lay on their side or back, with the head extended; others supported themselves on the side of the cage and extended their head and front legs; in either position they breathed with their mouth open. Respirations were dysp-

TABLE 1.—*Incidence of Gross and Microscopic Heart Lesions in the Rat After Treatment with 1-(3,4-Dihydroxy Phenyl)-2-Isopropyl Amino Ethanol HCl*

	Grade	Dose, Mg/Kg.																
		680	340	170	85	42.5	21.2	10.5	5.25	2.6	1.3	0.65	0.33	0.16	0.08	0.04	0.02	Control
Animals showing gross lesions, No.	0	1	1	1	--	--	--	--	--	2	--	4	6	5	6	10	10	10
	1	--	--	--	--	--	--	--	--	1	3	2	2	3	2	--	--	--
	2	1	2	1	--	3	3	6	6	5	6	4	2	1	2	--	--	--
	3	1	--	1	4	4	4	4	3	1	--	--	--	--	--	--	--	--
	4	7	7	7	6	3	3	--	1	1	--	--	--	--	--	--	--	--
Average severity		3.3	3.2	3.3	3.6	3.0	3.0	2.4	2.5	1.8	1.8	1.0	0.6	0.5	0.6	0	0	0
Heart *		--	--	--	0.607	0.557	0.593	0.535	0.519	0.481	0.427	0.464	0.410	0.429	0.432	0.452	0.473	0.342
Animals showing microscopic lesions, No.	0	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1	10
	1	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	4	--
	2	3	1	3	--	--	2	--	--	3	4	5	5	7	9	10	5	--
	3	2	4	--	--	2	1	6	6	4	4	4	3	3	1	--	--	--
	4	5	5	7	10	8	7	4	4	3	2	1	2	--	--	--	--	--
Average severity		3.2	3.4	3.4	4.0	3.8	3.5	3.4	3.4	3.0	2.8	2.6	2.7	2.3	2.1	2.0	1.4	0

* Expressed in gm. per 100 gm. body weight. Values are given only for those animals which survived 48 hr.

neic, irregular, and abdominal. Heart beats were strong at first and later weak and very rapid. At doses greater than 21.2 mg. per kilogram there was a reddish foamy exudate from the nostrils and corneal opacities. Many of these animals also had marked edema of the head, neck, chest, and front legs.

Marked lesions occurred outside the heart at dose levels of 170 mg. per kilogram and greater. These consisted of centrolobular liver necrosis and hemorrhage in 40% to 50% and cortical ischemia or necrosis of the kidney in 10% to 40% of the rats. In all animals, marked congestion with petechiae occurred in the abdominal viscera and leptomeninges and swelling of the brain was present. Pulmonary edema and hemorrhage, as well as hydrothorax, occurred in 40% to 90% of the rats receiving these higher doses.

At doses between 85 and 1.3 mg. per kilogram, no necrotic changes were observed outside the heart, but congestion of the liver, spleen, kidney, gastrointestinal tract, and leptomeninges, with cerebral swelling, was still observed in 30% to 100% of the animals at these doses. Pulmonary edema was occasionally observed in animals treated at doses between 85 and 21.2 mg. per kilogram.

At autopsy the hearts of most of the animals receiving DIH were obviously enlarged; when weighed this increase in size was found, as shown in Table 1, to be significant for those groups receiving doses of 5.25 mg. per kilogram or more. At dose levels lower than this the heart enlargement was less marked.

The cardiac necrosis was usually located at the apical area of the heart; however, when the lesions were severe the greatest part of the left ventricle and the apical portion of the right ventricle were also involved. Macroscopic lesions were occasionally seen in the auricles as indistinct, poorly demarcated, small, longitudinal, yellow streaks. The necrosis of the ventricles, which developed during the 48 hours of the experimental period, consisted of dry,

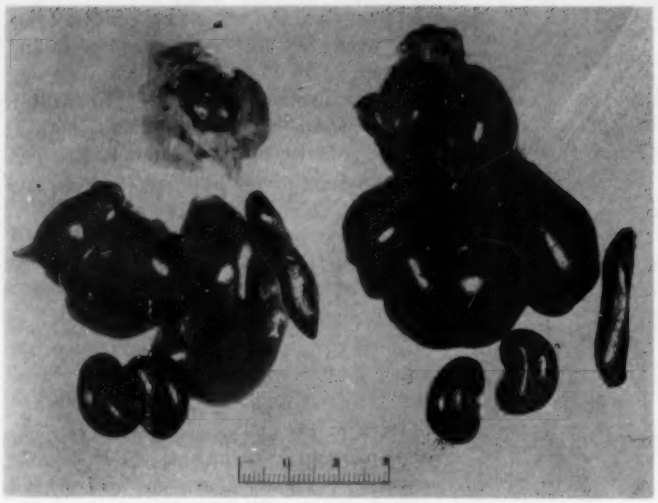

Fig. 1.—Heart, lungs, spleen, liver, and kidneys (left) from a control rat and (right) after 85 mg. of isoproterenol hydrochloride per kilogram subcutaneously on two consecutive days. Necrosis is evident at the apex of the heart, with severe congestion of the lungs and abdominal viscera.

granular, friable, pale grayish-yellow areas, occasionally surrounded by dilated veins and hemorrhage. The cut surface of large infarct-like lesions was homogeneous; however, smaller lesions revealed a mottled or striated cross section. The areas of necrosis of the ventricular wall appeared to be thin and bulging, compared with the surrounding areas which were thickened.

The group treated with 85 mg. per kilogram showed the most marked cardiac enlargement (0.607 gm/100 gm. of body weight). This group revealed also the severest cardiac necrosis, with an average grade of severity in the gross of 3.6, as illustrated in Figure 1. Nine of the rats in this group had infarct-like myocardial lesions involving the apex, the lower part of the left ventricle, the interventricular septum, and occasionally the right ventricle. Groups treated with higher doses had relatively less-pronounced cardiac changes, but 40% to 50% of these animals died after only one treatment with DIH, and the time lapse was not sufficient for development of lesions of maximal severity. Animals which survived long enough, however, manifested large infarcts of hemorrhagic character; in four animals mural thrombi were also seen in acute aneurysms of the left ventricle. The groups treated with doses between 42.5 mg. and 5.25 mg. per kilogram had 100% incidence of cardiac necrosis, with a decrease in size as the dose of the drug was reduced. Rats treated with doses of 2.6 mg. to 0.08 mg. per kilogram also manifested infarct-like apical necrosis; however, gross necrosis was observed only in 20% to 50%. In other instances there was a mottling of the distal portion of the left ventricle, caused by intermingled pale and dark red streaks. Only the groups treated with the 0.04 and 0.02 mg. per kilogram doses and the control group were free from gross cardiac lesion.

Fig. 2.—Necrosis involving the whole thickness of the left ventricle, causing acute aneurysm and mural thrombus. Rat was treated with 680 mg. per kilogram of DIH. Cason's trichrome; × 20.

Microscopic Picture of Heart Lesions.—Microscopically the necroses were focal, confluent, or massive and infarct-like. The latter were found most frequently in the apex at dose levels from 680 mg. to 0.33 mg. per kilogram. The necrosis often involved the whole wall (Fig. 2), but in other cases at lower doses only a partial thickness was necrotic. In the latter case the subendocardial sheet was more involved than the subepicardial portion of the myocardium. At a dose of 85 mg. per kilogram massive necrosis was observed in the apex and/or the papillary muscles and adjacent subendocardial portions of the left ventricle and less frequently the right ventricle in nine rats. The cardiac infarcts, observed grossly, were found on microscopic examinations to be either massive or confluent necrosis. Confluent necrosis differs from the massive lesion in the respect that some relatively unaffected muscle fibers are present in the necrotic areas. Generally muscle fibers lying near the capillaries showed less histolysis than muscle bundles which were located further from the blood supply. This characteristic of the lesion gave a variegated appearance to the confluent necrosis. It had the same localization as the massive lesion; however, unlike the massive infarct-like necrosis, confluent necrosis was also found in the auricles. It was seen in all rats treated at doses from 85 mg. to 10.5 mg. per kilogram. At higher doses the focal necroses were located in the basal portion of the left ventricle, the right ventricle, and the auricles. They may show the same histological characteristics as the confluent lesion; however, the necrosis of the muscle fibers was less pronounced and the reactive changes predominated. At lower dose levels the focal lesions were observed at the apex and papillary muscle of the left ventricle. They were composed of edema, histiocytes, swelling, and proliferation of the fibroblasts, but regressive changes of the muscle fibers were generally mild or absent.

Components of Myocardial Lesions.—The heart lesions had histological components which lent themselves to being grouped into

TABLE 2.—*Incidence of Histological Components of the Myocardial Lesions*

	Dose, Mg/Kg.																
	680	340	170	85	42.5	21.2	10.5	5.25	2.6	1.3	0.65	0.33	0.16	0.08	0.04	0.02	Control
Mottled staining	9	10	10	10	10	10	10	10	7	6	8	8	8	4	1	;	;
Vacuolar degeneration	;	;	3	6	2	2	1	1	;	;	;	;	;	;	;	;	;
Fatty degeneration	7	8	8	10	8	7	1	1	;	;	;	;	;	;	;	;	;
Granular disintegration	10	10	10	10	10	10	10	10	6	7	6	6	5	2	;	;	;
Hyaline necrosis	7	8	9	10	10	9	7	8	4	3	4	2	;	;	;	;	;
Capillary dilatation	8	10	9	9	9	3	3	2	3	2	;	2	1	;	;	;	;
Interstitial edema	9	10	9	10	10	10	10	8	10	8	8	4	5	4	3	3	;
Sequestrating edema	1	3	5	6	5	2	4	2	2	1	1	;	;	;	;	;	;
Leukocytes	9	8	8	10	3	2	3	4	;	;	;	;	;	;	;	;	;
Histiocytes	4	2	5	10	10	8	10	10	8	7	7	6	6	4	4	1	;
Fibroblastic swelling	5	3	4	10	10	8	10	10	10	10	10	10	10	10	10	9	;
Fibroblastic proliferation	2	1	3	9	10	8	10	10	6	5	5	4	2	2	3	3	;

two classifications: first, regressive lesions of the myocardial fibers, and, second, reactive inflammatory changes of the stroma. They occurred, as shown in Table 2, in combinations which allowed correlations to be drawn between the dose of drug administered and the histological features of the cardiac necrosis.

1. Regressive Changes of the Myocardial Fibers: The mildest lesion of the muscle fibers was the eosinophilia of the cytoplasm. The involved fibers were swollen, revealing segmentation and fragmentation. They gave a weak P. A. S. reaction and stained pink with Cason's trichrome. Some groups of muscle fibers showed this change, while neighboring bundles were preserved. The alternating staining characteristics of these areas caused a mottled appearance in the myocardium. The nuclear staining and structure of the muscle fibers was preserved. This change was observed in all animals given doses as low as 5.25 mg. per kilogram, and 80% of the animals had this lesion at a dose level of 0.16 mg. per kilogram. At higher doses mottled, eosinophilic muscle bundles were found at the margin of necrotic areas. However, at lower doses (2.6-0.08 mg. per kilogram) they were the most prominent components of the heart lesion.

At dose levels from 170 to 5.25 mg. per kilogram, 10% to 30% of the rats showed vacuolar degeneration of the muscle fibers. At a dose of 85 mg. per kilogram this change occurred in six animals. The muscle fibers involved were ballooned out, and large vacuoles, as shown in Figure 3, pushed the swollen nuclei to one side of the cytoplasm. The vacuoles did not stain with Sudan IV in frozen sections and gave a negative P. A. S. reaction. This change occurred in small groups of muscle fibers, located most frequently at the apex or beneath the endocardium of the left ventricle, together with extensive hyaline necrosis.

In a severer stage of regressive muscle changes the swollen fibers disintegrate into small granules; the outlines of the muscle fibers are lost, and the nuclear staining disappears. This granular disintegration was found in 100% of the animals receiving doses of 5.25 mg. per kilogram or greater, with a decreasing incidence down to doses of 0.08 mg. per kilogram.

The most characteristic lesion of the muscle fibers was hyaline necrosis. They became homogeneous, strongly eosinophilic, and P. A. S.-positive (Fig. 4), and stained deep red with Cason's trichrome. Striation of the muscle fibers was lost, and the nuclei were either shrunken or disappeared. The

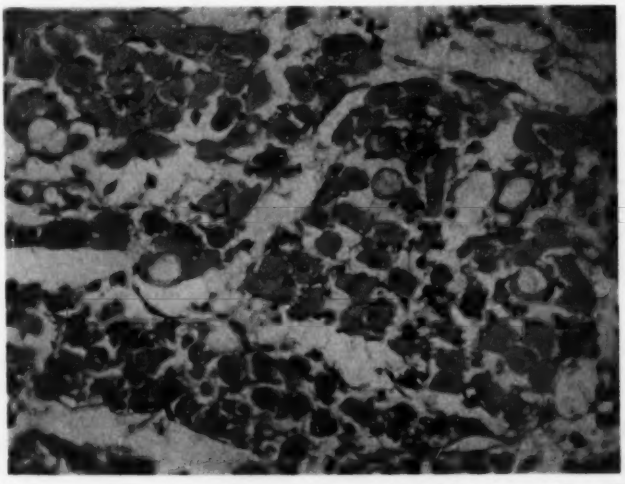

Fig. 3. — Vacuolar degeneration of muscle fibers adjacent to an area of hyaline necrosis. Periodic acid Schiff; × 300.

Fig. 4.—Hyaline necrosis of muscle fibers separated from normal fibers by mucoid edema. Periodic acid Schiff; × 120.

necrotic fibers also stained with Sudan IV in frozen section. Fatty changes of the muscle were most prominent at the higher dose levels. Hyaline necrosis was present in 70% to 100% of the hearts of the animals receiving doses of 5.25 mg. per kilogram or greater and was the primary component of the massive infarct-like necrosis. At lower doses hyaline necrosis was also observed in confluent lesions. It did not occur at dose levels below 0.33 mg. per kilogram.

2. Reactive Changes of Stroma: The reactive changes which occurred around the involved muscle fibers consisted of capillary dilatation, interstitial edema, leukocytic and histiocytic infiltration, and fibroblastic changes. Widespread capillary dilatation was observed in 80% to 100% of the animals receiving doses of 85 mg. per kilogram or greater. At lower doses the dilatation was less frequent and restricted to the areas showing regressive changes. As a result of increased capillary permeability edema developed, which diffused throughout the stroma, making it appear loose. The edema, in more pronounced cases, separated the muscle fibers. This sequestrating type of

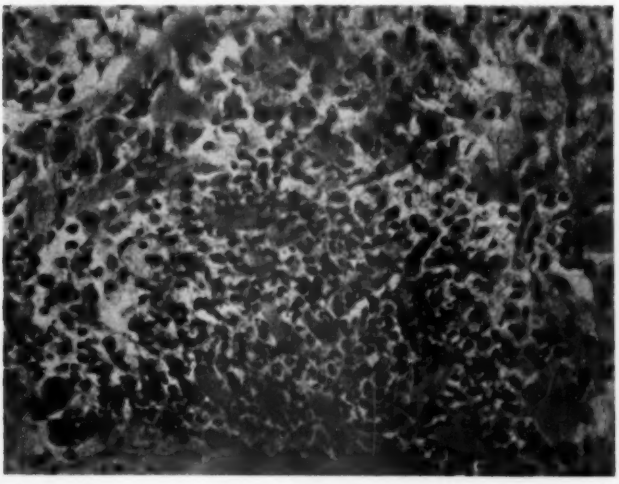

Fig. 5.—Leukocytic reaction at the margin of an area of necrosis. Hematoxylin and eosin; × 300.

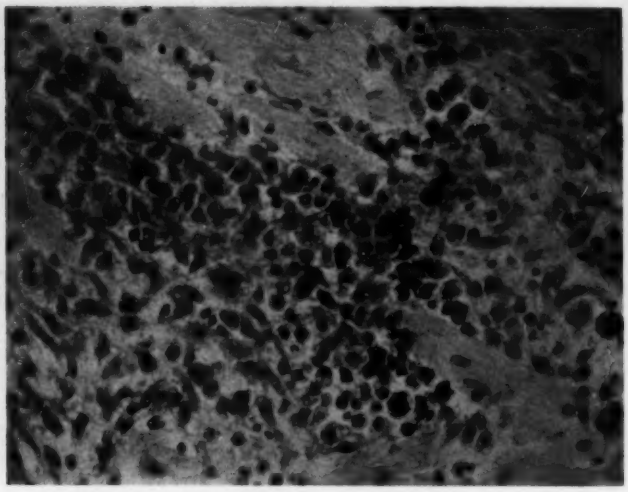

Fig. 6.—Histiocytic foci in the myocardium. The dark granules in the cells are hemosiderin. Hematoxylin and eosin; × 300.

edema was more marked at dose levels of 85 and 42.5 mg. per kilogram. The irregular spaces were generally located in the left ventricle, parallel with the muscle fibers, isolating some subendocardially located bundles from the remaining part of the myocardium. The edematous areas were relatively acellular; however, in dose levels from 85 to 5.25 mg. per kilogram they contained many swollen, star-shaped cells. In such cases the intracellular fluid stained light blue with hematoxylin and gave a positive P. A. S. reaction. In two cases (at doses 21.2 and 10.5 mg. per kilogram) some fibrin threads were also observed in the edematous fluid.

The inflammatory cells, forming a reaction around the involved muscles, were leukocytes and mononuclear cells. Leukocytic reaction was frequent in animals treated with higher doses (Fig. 5). It decreased abruptly in animals treated with less than 85 mg. per kilogram. Leukocytes were not found in appreciable numbers around necrotic foci in animals treated with 2.6 mg. per kilogram or less. The mononuclear cells were mostly histiocytes (Fig. 6). These contained occasional fat droplets or

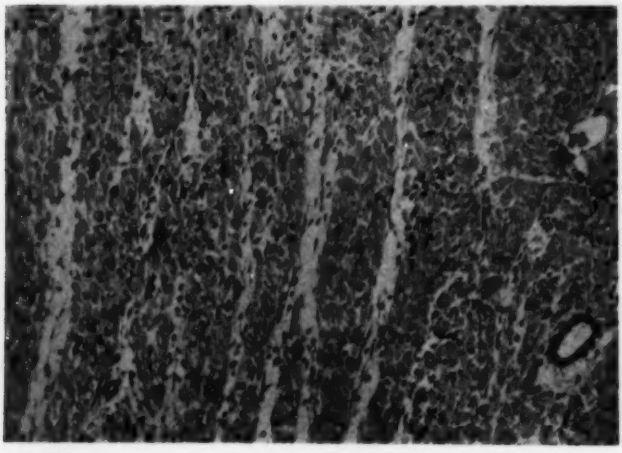

Fig. 7.—Swelling of fibroblasts between the muscle fibers. Hematoxylin and eosin; × 120.

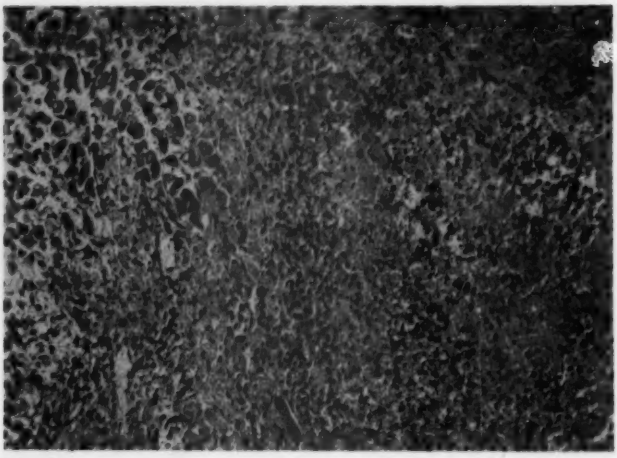

Fig. 8.—Fibroblastic proliferation at the border of an area of massive necrosis. Hematoxylin and eosin; × 120.

hemosiderin pigment. There were also some lymphocytes and mast cells. At doses below 85 mg. per kilogram the leukocytic reaction was replaced by histiocytes, which were pronounced down to doses of 0.16 mg. per kilogram. At doses of 0.08 and 0.04 mg. per kilogram the focal changes of the myocardium contained histiocytes.

The swelling and proliferation of fibroblasts became marked at 85 mg. per kilogram. The fibroblasts, which are normally flat and hardly visible among the muscle fibers, became swollen, oval, or polygonal (Fig. 7). This change was widespread and was seen even at the lowest doses, when other changes were indistinct or absent. The proliferation of fibroblasts was pronounced around the margins of massive necroses. (Fig. 8). The endothelial cells of the endocardium showed similar changes. Along with the fibroblasts newly formed capillaries penetrated into the necrotized areas, introducing the process of organization. Between dose levels of 85 mg. and 2.6 mg. per kilogram many mitotic figures and multinucleated fibroblasts could be seen (Fig. 9). The cytoplasm of these cells was basophilic. Among them there were groups of cells with elongated nuclei and scanty cytoplasm. The chromatin was accumulated longitudi-

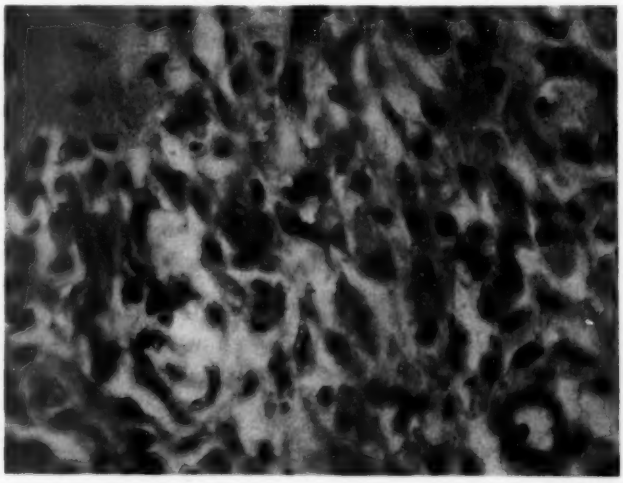

Fig. 9.—Fibroblasts, revealing multiple nuclei and mitotic figures. Hematoxylin and eosin; × 500.

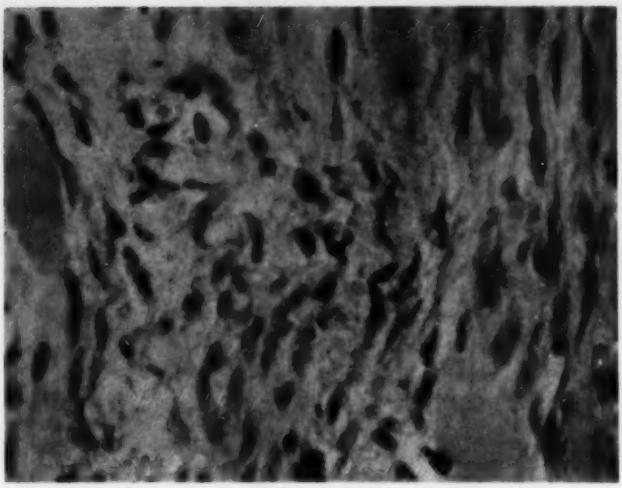

Fig. 10. — Large numbers of Anitschkow myocytes between necrotic muscle fibers. Hematoxylin and eosin; × 500.

nally at the center of the nuclei and formed fine dentation in the direction of the nuclear membrane (Fig. 10). These cells were identical with the Anitschkow myocytes. The proliferation of the fibroblasts was also observed independently from necrosis. At lower doses (2.6 to 0.02 mg. per kilogram) the foci in the myocardium were composed mostly of fibroblasts. In three instances, at dose levels of 21.2 and 5.25 mg. per kilogram, we observed well-circumscribed subendocardial granulomata, whose necrotic centers were surrounded by swollen fibroblasts, giant cells resembling Aschoff giant cells, and histiocytes.

Coronary artery. Apart from the swelling of the wall of the arterioles inside the areas of massive necrosis no changes were observed in the coronary arteries. In one animal only, treated with 680 mg. per kilogram, hyalin thrombi were seen in some small coronary branches, lying inside an apical infarct.

Valves. Our attention was not directed in the present study toward lesions of the valves. Occasionally a swelling of the bicuspid and aortic valves was observed. In four animals, treated with high doses, scattered foci composed mostly of mononuclear cells were observed in the bicuspid valve.

Pericardium. The endothelial cells covering the infarcted areas were swollen. Some fine threads of fibrin, forming villi on the pericardium, could also be seen occasionally.

Comment

Our experimental results clearly demonstrate that DIH in a wide range of doses could produce massive cardiac necrosis closely resembling the experimental myocardial infarct which is seen following coronary artery ligation or the myocardial infarct of the human. The fully developed necrosis, like the ischemic necrosis of the myocardium, was localized generally in the apex, less frequently in the papillary muscle, and occasionally in the right ventricle. Histologically the muscle bundles showing granular disintegration or hyalin necrosis were surrounded by a leukocytic demarcation zone. Widespread capillary dilatation and marked edema were observed. Similar changes were reported by Johns and Olson [9] in rats after ligation of the coronary artery. The rapidity of the fibroblastic proliferation and the great number of Anitschkow myocytes [39] seem to be remarkable. Rats killed 48 hours after the first injection revealed a wide zone of fibroblasts around the necrotic muscle fibers. The penetration of the fibroblasts into the necrotic area was followed by an active budding and ingrowth of newly formed capillaries. Fibroblastic foci were

also observed independently from manifest myocardial necrosis. According to Mallory, White, and Salcedo-Salgar,[40] in human cases the proliferation of fibroblasts and the penetration of newly formed blood vessels into the infarcted area begin about the fourth day after coronary occlusion. Karsner and Dwyer[41] reported that in myocardial infarction of the dog the proliferation of fibroblasts starts after 24 hours, following the ligation of the coronary artery, and is marked after 48 hours. Consequently, the rat reacts in a manner similar to the dog, suggesting a greater reactivity of repair processes in these species.

Though the cardiac necrosis produced by DIH corresponds in gross and histological characteristics to that of the ischemic necrosis of man and experimental animals, no changes were observed which suggested a disturbance of coronary circulation. Except in one case, we did not observe any organic lesions in the coronary artery. Since according to Denison, Bardhanabaedya, and Green[42] DIH causes dilatation of the coronary vessels, the basis of the myocardial necrosis does not appear to be vascular spasm or occlusion.

It has been reported[43,44] that DIH administered intravenously to the dog caused a marked fall in the blood pressure and increased the rate and amplitude of the heart beat. According to Solbach,[15] acute coronary insufficiency arises when the balance between the oxygen need and blood supply of the myocardium is disturbed. The immediate result will be hypoxemia of the myocardium, followed in severe cases by myocardial necrosis. It has been shown[14] that in collapse of different origin the coronary circulation is diminished, causing multiple myocardial necroses. Similar necrosis was also produced by epinephrine infusion,[18] which caused myocardial ischemia by increasing the oxygen requirement of the heart muscle, and so in spite of coronary vasodilatation a relative insufficiency of oxygen was produced.[45] In the light of these studies it seems probable that a similar mechanism may play a role in the development of the cardiac necrosis produced by DIH.

This theory is strengthened by the results obtained after low doses of DIH. Doses lower than 5.25 mg. per kilogram seldom produced massive, infarct-like necrosis, but rather multiple disseminated necrosis developed, which closely resembled that obtained by the German authors. Nevertheless, DIH, unlike epinephrine or collapse, also produced massive infarct-like necrosis of the myocardium. Consequently, it possesses some specific characteristic. DIH, like collapse, diminishes the blood supply of the myocardium by lowering the blood pressure. Like epinephrine, DIH has also a direct cardiac action, increasing the oxygen need of the heart muscle. These combined effects of DIH could explain the production of isolated gross cardiac necrosis in all animals at doses as low as 1/500 of the L. $D_{.50}$ The morphological effects of DIH on the heart, which have not hitherto been disclosed[43,46] and which are produced at doses below those that cause any other toxic manifestations, suggest the use of this drug as a means of producing experimental cardiac necrosis. Since relative hypoxemia of the heart muscle can cause myocardial infarction in man without coronary occlusion,[47-50] our findings with DIH may have considerable therapeutic importance.

Summary

It is shown that isoproterenol (1-[3′,4′-dihydroxyphenol]-2-isopropylaminoethanol) hydrochloride is capable, when administered subcutaneously to the rat, of producing gross and microscopic myocardial necrosis. There is a close correlation between the dose injected and the degree of severity of the necrosis, which makes possible the production of standardized myocardial lesions.

On the basis of the localization and histological characteristics of the myocardial necrosis produced by this drug and the similarity to ischemic necrosis produced by other techniques we tentatively conclude that

the lesions in both cases have a common pathogenetic mechanism. The pharmacologic properties of this drug which would contribute to the production of these lesions are discussed.

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Pancreatic Adaptation to Diabetogenic Hormones

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The syndrome of diabetes mellitus, which is characterized by persistent hyperglycemia with or without glycosuria, results from a derangement in the mechanism of blood sugar homeostasis. This defect may appear spontaneously as a result of a disequilibrium between the rates of production of insulin, on one hand, and those of growth hormone or adrenal steroids, on the other.[1-4] However, the pancreas is not static in its insulinogenic activity,[5] and so a marked endogenous increase in these anti-insulin factors, as in Cushing's disease[6] or acromegaly,[7] does not always lead to diabetes. Experimentally, gradual adaptation of the organism to the diabetogenic action of exogenous hormones can usually be demonstrated. Thus, the temporary diabetes induced by adrenal steroids or adrenocorticotropin administration in the intact animal is characterized by the gradual development of glycosuria, which then gradually declines, only to become reestablished when the dosage is increased.[8-11] The fact that this adaptation to a diabetogenic stimulus is frequently overcome when the pancreatic reserve is reduced either surgically[12-14] or chemically[15] suggests that increased insulinogenesis is the mechanism involved. This hypothesis is supported by the fact that permanent metasteroid diabetes may be produced in the partially pancreatectomized dog, whereas the normal dog is resistant to the action of cortical extracts.[16]

The present study concerns the structural alterations in the pancreas of rabbits and dogs during treatment with adrenal steroids, growth hormone, and glucagon, either alone or in combination, and their correlation with the physiologic observations.

Material and Methods

A. Rabbit Experiments.—The study was carried out on 68 New Zealand White rabbits of either sex, weighing from 2.5 to 4 kg. Twelve received 1 mg. of cortisone acetate* per kilogram intramuscularly daily for 18 to 72 days. Thirty-six were given daily 4 mg. of cortisone acetate per kilogram intramuscularly during the first 21 days and then 8 mg. per kilogram daily thereafter for up to 60 days. Eighteen received subcutaneously 1 mg. of crystalline glucagon† suspended in corn oil per kilogram three times daily, at 10 a. m., 4:30 p. m., and 12 midnight, and in addition 1 mg. of cortisone acetate per kilogram in aqueous suspension intramuscularly daily. Twelve untreated rabbits served as controls. Each animal was kept in a metabolic cage and received Purina Rabbit Chow and water ad libitum. Blood for glucose determinations was drawn twice daily in the nonfasting state from the marginal ear veins.

B. Dog Experiments.—A group of 30 mongrel dogs of either sex, weighing from 12 to 16 kg., were 50% partially pancreatectomized. On or after the 14th postoperative day, 18 received 3 mg. of growth hormone per kilogram‡ daily subcutaneously for a period of 1 to 35 days. The other 12 served as controls. All dogs were kept in metabolic cages and received a high-carbohydrate diet, consisting of mashed potatoes, meat, meat gravy, and 5% sucrose solution ad libitum instead of drinking water. Blood for glucose determination was withdrawn each morning from the leg veins.

The blood sugar determinations were carried out by the Nelson-Somogyi micromethod.[17] All animals

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† Crystalline Glucagon (Lot No. 258-234-B-54-2) was supplied by the Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, by Dr. W. R. Kirtley.

‡ Growth hormone (R50109) was a gift from the Endocrinology Study Section, National Institutes of Health.

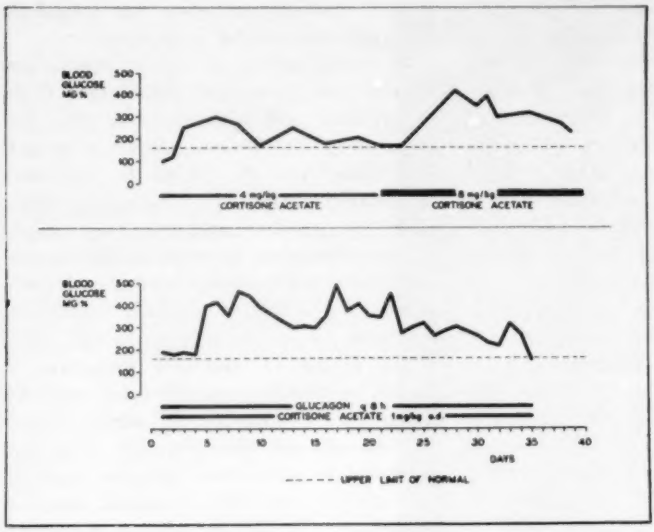

Fig. 1.—The upper curve shows the average daily blood glucose concentration of a representative rabbit, treated with 4 mg. daily of cortisone acetate per kilogram for 21 days and 8 mg. per kilogram daily thereafter. The lower curve shows the average daily blood glucose concentration of a representative rabbit, treated simultaneously with 1 mg. of crystalline glucagon in corn oil t. i. d. and 1 mg. daily of cortisone acetate per kilogram.

were killed by overdosage with pentobarbital (Nembutal), and pancreatic tissue was placed immediately in Zenker-formol solution. The tissue blocks were embedded in paraffin, and sections, stained by a modification of the aldehyde fuchsin technique,[18] a modification of the Masson trichrome technique,[19] and the periodic acid-Schiff technique with a trichrome counterstain[20] controlled by diastase digestion. In addition, tissue was embedded in Carbowax and stained simultaneously for carbohydrate with the periodic acid-Schiff technique and for fat with Sudan IV in propylene glycol.

Results

A. Rabbit Experiments.—The untreated rabbits showed no significant differences between the morning and afternoon blood sugar values, which varied between 93 and 158 mg. %, with a mean of 127 mg. %. A nonfasting blood sugar value of greater than 160 mg. % was considered a significant elevation. The greatest degree and duration of hyperglycemia were developed in those rabbits treated simultaneously with cortisone and glucagon. In these animals, the morning dose of glucagon produced a marked hyperglycemic response, which was fairly well sustained until the afternoon. The morning values were frequently within normal limits during the early stages of treatment. Thereafter they increased to as high as 440 mg. %. The afternoon values were elevated from the beginning. This elevation became extreme as treatment continued, with occasional values reaching 840 mg. %. After six weeks, the morning glycemic levels were frequently less than prior to that time and tended to return to normal (Fig. 1).

Rabbits treated with cortisone showed no differences between the morning and afternoon blood sugar values. With a dose of 1 mg. per kilogram the glycemic level rarely exceeded the normal and varied up to 173 mg. %. It tended to be higher during the first few weeks after treatment was initiated, and then it declined to average normal values. Rabbits treated with the larger and increasing dosages of cortisone showed, in general, two peaks of glycosuria. The first peak was reached between the third and fifth days after treatment was initiated with 4 mg. of cortisone per kilogram daily. After that time the blood sugar tended to decline, reaching its nadir within three weeks. When the dose of cortisone was doubled at 21 days a secondary rise in glycemic level was usually obtained, which then again tended to decline. With these cor-

tisone dosages the maximum blood sugar level varied up to 375 mg. % (Fig. 1).

Morphologically, degranulation of β-cells was frequently observed within 48 hours after the start of hormone treatment. Its extent seemed related to the severity of the hyperglycemia. Thus, the rabbits treated with 1 mg. of cortisone per kilogram displayed only occasional β-cell degranulation, while the other two groups usually showed well-marked or complete β-cell degranulation. A similar relationship was noted between hyperglycemia and glycogen infiltration. This lesion appeared first in ductular epithelium and then in β-cells. Its extent was related to the severity and the duration of diabetes. Sudan IV-positive material was not found in vacuolated rabbit ductular epithelium or in β-cells. Two types of ductules could be differentiated in the severely diabetic rabbits. There were occasional nonvacuolated hyperplastic ductules, which were frequently contiguous with or within islets. The other ductules were all markedly vacuolated and frequently were also intrainsular in position.

Increased mitotic activity of β-cells was seen in animals treated with larger doses of cortisone and with cortisone plus glucagon (Fig. 2). This was observed in animals killed from the 4th to the 10th days of treatment and only very rarely thereafter. In animals treated over long periods of time the islets were frequently hyperplastic and had irregular contours. Hyperplastic nonvacuolated ductular epithelium was seen contiguous with or within islets (Figs. 3 and 4), and new formation of β-cells by primitive intermediates could be traced in the longest-term rabbits which received cortisone plus glucagon. This was evidenced by individual sparsely granular cells within cords of hyperplastic ductular epithelium (Fig. 4). In addition, there were intrainsular young β-cells, which were in direct contiguity with duct epithelium and with surrounding more mature β-cells. The immature cells were large, with vesicular

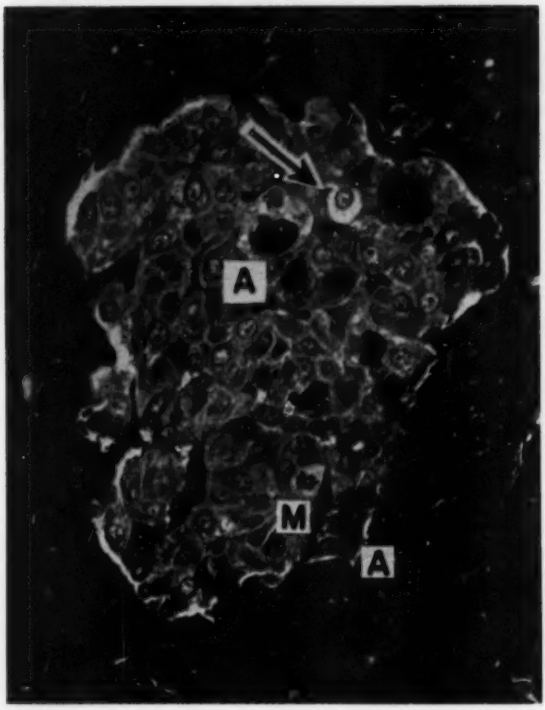

Fig. 2.—Islet from pancreas of rabbit treated with 4 mg. of cortisone acetate per kilogram for six days. There is extensive degranulation of the β-cells as well as a mitotic figure present (*M*). In addition, there is a cell with vacuolated non-glycogen-containing cytoplasm and intact nucleus (arrow). The α-cells appear dark (*A*). Periodic acid-Schiff-trichrome stain; × 375.

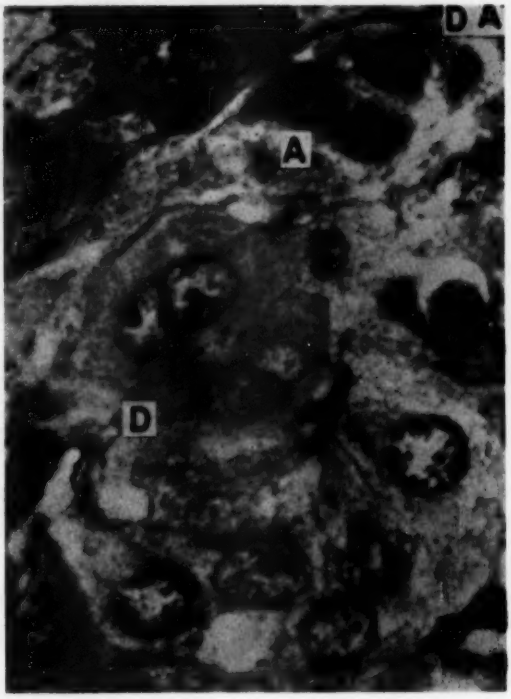

Fig. 3.—Pancreas of a rabbit treated simultaneously for 51 days with glucagon and cortisone, showing a proliferating nonvacuolated ductule (*D*), with surrounding islet tissue. Also seen are a granulated (*A*) and a partly degranulated (*DA*) α-cell. Modified Masson trichrome stain; × 1,300.

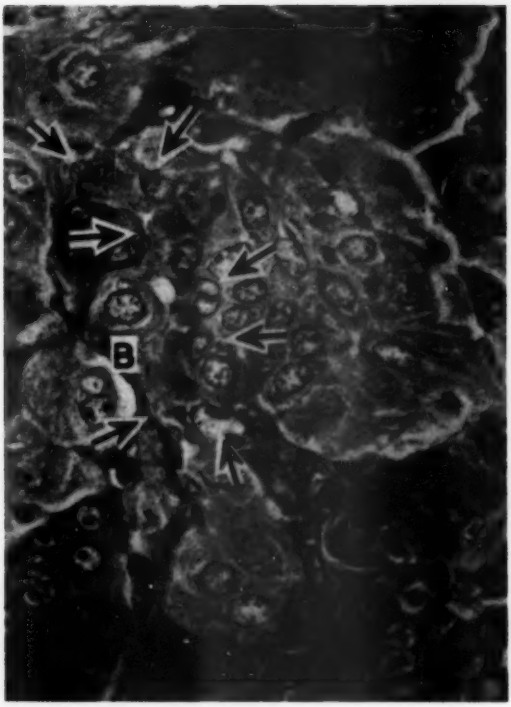

Fig. 4.—Pancreas of rabbit treated with glucagon and cortisone simultaneously for 104 days, showing an intrainsular proliferating ductule (arrows), with a primitive β-cell (*B*) originating from it. Modified Masson trichrome stain; × 520.

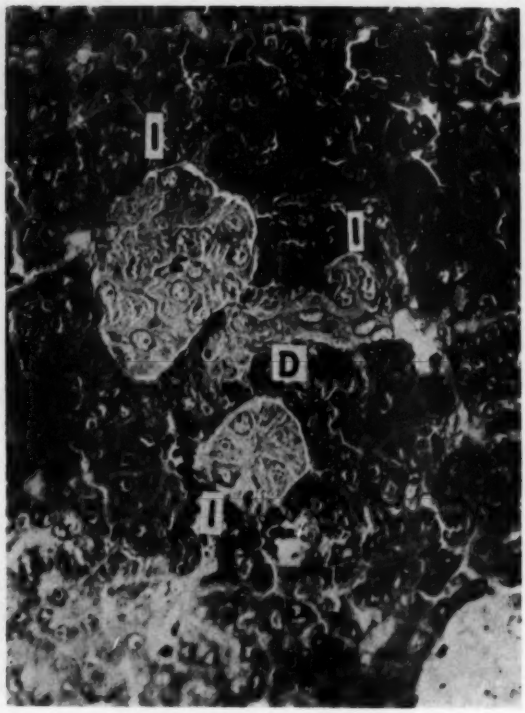

Fig. 5.—Pancreas of same rabbit as in Figure 4, showing a hyperplastic ductule (*D*), with attached newly formed islets (*I*), composed of β-cells only. Modified Masson trichrome stain; × 375.

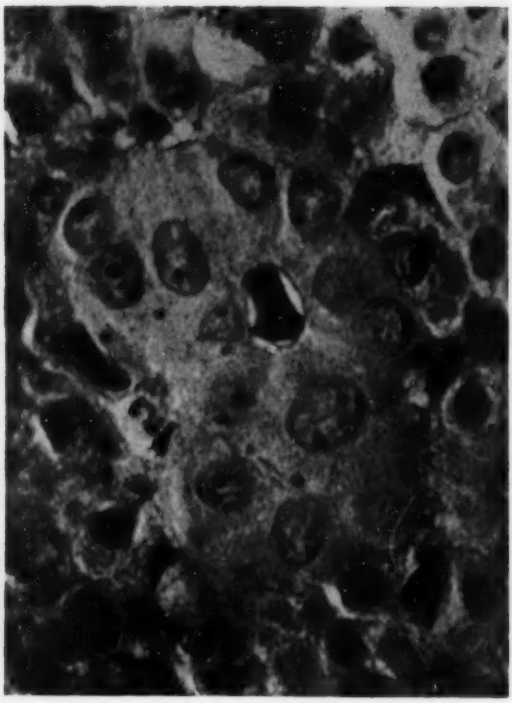

Fig. 6.—Pancreas of same rabbit as in Figure 4, showing an acinar arrangement of β-cells with Schiff-positive material in lumen. Periodic acid-Schiff stain; × 1,050.

nuclei and sparsely distributed aldehyde-fuchsin-positive granules in the cytoplasm. Adjacent older β-cells were smaller and frequently degranulated and showed aldehyde-fuchsin-positive cell borders. Occasional young small islets were observed, which in appendage-like fashion were loosely attached to a hyperplastic duct (Fig. 5). Also an acinar arrangement of β-cells around a central lumen, which contained Schiff-positive material, was occasionally seen (Fig. 6). There were also, in some instances, isolated well-granulated β-cells seen within groups of degranulated older β-cells with aldehyde-fuchsin-positive borders. These isolated granulated β-cells are thought to represent a new generation, as compared with the older β-cells which have become degranulated under the influence of hormonal treatment. There were additional findings in the animals treated with large doses of cortisone, which have been previously described.[21-24] These included lobules or parts of lobules, consisting of proliferating dilated ductules containing inspissated secretion, dedifferentiated acinar tissue, and comparatively intact islets. In addition, there was frequent peripancreatic fat necrosis noted. After treatment with cortisone plus glucagon there was α-cell degranulation, as described elsewhere.[25]

B. Dog Experiments.—Within 48 hours after growth hormone administration was begun the morning blood sugar values had usually risen to over 200 mg. %. By the fourth or fifth days the glycemic level reached approximately 300 to 500 mg. %. This level was maintained until the animals were killed. Morphologically, there was progressively greater β-cell degranulation which became pronounced after 48 hours. At this time there was also increased ductular glycogen infiltration, which, as the experiment was continued, also became progressively accentuated. β-Cell glycogen infiltration was first apparent at four days and also gradually became more extensive. At about 9 or 10 days, in animals with

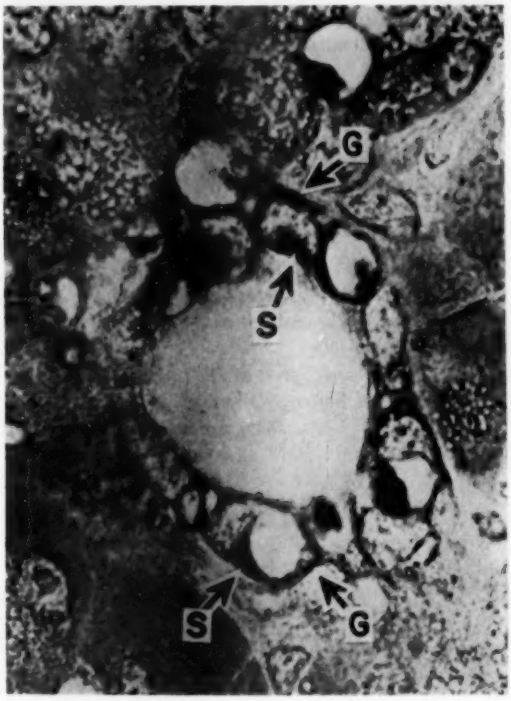

Fig. 7.—Pancreas of a 50% partially pancreatectomized dog, treated with 3 mg. of growth hormone per kilogram daily for 11 days. There is a marked vacuolation of duct epithelium and of centroacinar cells, which show frequently both Schiff-positive (*G*) and sudanophilic (*S*) material in the same cell. Carbowax-embedded, periodic acid-Schiff-Sudan IV stain; × 1,300.

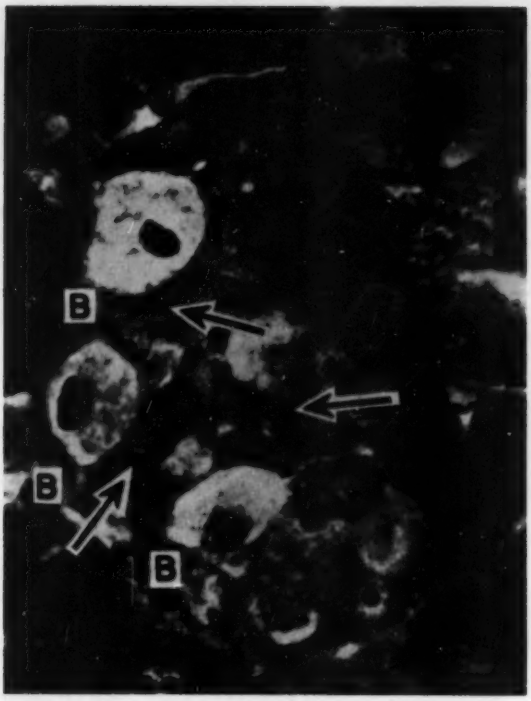

Fig. 8.—Islet from pancreas of 50% partially pancreatectomized dog, treated with 3 mg. of growth hormone per kilogram daily for six days. Three ballooned-out (*B*) cells with reticulated partly vacuolated cytoplasm and pyknotic nuclei are seen. The β-cells are degranulated and show glycogen deposits (arrows). Periodic acid-Schiff-trichrome stain; × 1,300.

extensively vacuolated ductular epithelium, Sudan-positive material was present in addition to periodic acid-Schiff-positive material (Fig. 7).

An additional alteration was seen in scattered individual intra- or sometimes extra-insular cells. These cells were ballooned-out and showed reticulated partly vacuolated cytoplasm but were neither Schiff- nor Sudan IV-positive. The nuclei showed gradations of pyknosis suggestive of early loss of cellular viability. After 14 to 21 days of treatment with growth hormone, islet tissue was difficult to find in the pancreas. Occasional residual islets were composed mostly of α-cells with agranular cells of unidentifiable antecedents (Fig. 9). In the dog pancreas studied no evidence was found of neogenesis of β-cells either from ductular epithelium, from acinar tissue, or from other cell types. Occasional mitoses were found in β-cells; however, equal numbers of mitoses were also found in duct epithelium as well as in acinar cells.

Comment

The present study demonstrates that, in the rabbit, at any one hormone-dosage level the degree of diabetes wanes as treatment is prolonged. Contrariwise, in the dog receiving an adequate dosage of growth hormone the diabetes is maintained and may persist even when the hormone administration is discontinued. The serial morphologic changes in the pancreas seem to go hand in hand with the changes in metabolic pattern. Soon after initiation of hormone administration there is degranulation and increased mitotic activity of the β-cells. These two phenomena are morphologic indicators of increase in functional activity of β-cells, with resultant increase in insulin output. They serve as compensatory mechanisms to restore blood sugar homeostasis. As hormone treatment is prolonged a dichotomy develops between the evolution of events in the two species. In the dog there is destruction of pancreatic β-cells and ensuing

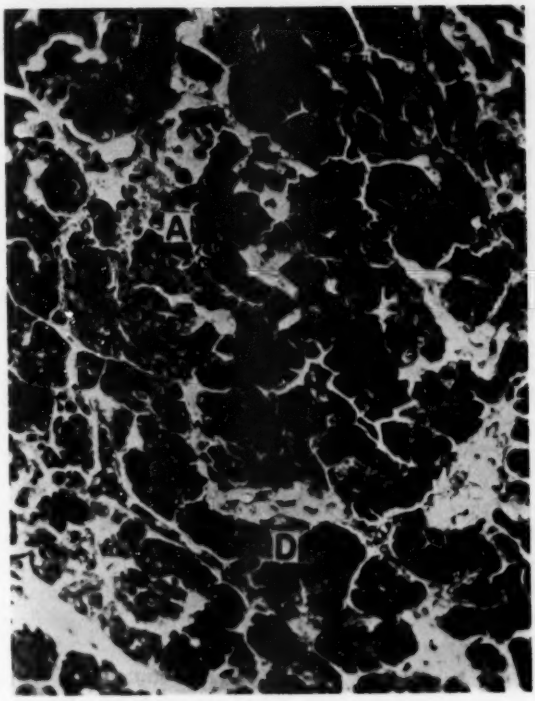

Fig. 9.—Pancreas of 50% partially pancreatectomized dog, treated with 3 mg. of growth hormone per kilogram daily for 21 days. There is marked ductular (*D*) vacuolation and loss of β-cells. A residual islet is composed mostly of α-cells (*A*). Periodic acid-Schiff-trichrome stain; × 250.

permanent metahypophyseal diabetes. In the rabbit, on the other hand, neogenesis of β-cells via primitive precursors is traceable from pancreatic duct epithelium and eventually the diabetes ameliorates.

Previously it has been shown [26,27] that the puppy and the pregnant bitch resist the diabetogenic action of growth hormone. This difference in response of the immature, as compared with the mature dog, is thought to reflect differences in ability for formation of new β-cells.

These experimental findings might have application to the human, whose pancreas probably responds somewhat like that of the rodent rather than that of the canine. It has been shown that there may be hyperplasia of islet tissue in some forms of adult diabetes [28,29] as well as in infants of prediabetic mothers.[30-32] In both instances it is probably a compensatory response. Furthermore, prolonged human diabetes due to adrenocortical tumor [33] or pheochromocytoma [34] has been cured by surgery. This observation suggests that in man, as in the rodent, prolonged diabetes may occur without damage to the pancreas sufficient to maintain the diabetic state after removal of the etiologic agent.

The vacuolization and destruction of canine β-cells during diabetes has previously been ascribed to inbibition of fluid by these cells and termed "hydropic degeneration." [35-39] More recently it was demonstrated that in both canine and rodent diabetes vacuolated pancreatic ductular epithelium and β-cells contain glycogen.[40] The concept that this glycogen infiltration in the β-cells is the pathogenetic mechanism which leads to their destruction seemed, however, to be negated by the finding that the ductular glycogen infiltration precedes that in the β-cells.[40-44]

In the present study, in accord with a previous report,[39] fat was sometimes also found in vacuolated duct epithelium in the dog. There were also additional ballooned-out intraislet cells which with present tech-

niques did not stain for either fat or glycogen. These cells showed gradations of nuclear pyknosis, indicative of severe cellular damage, and probably represented the first morphologic evidence of β-cell destruction. This finding supports the hypothesis that glycogen infiltration of β-cells is probably not a precursor of β-cell destruction.[21,22,41-44]

The results in the rabbit are in accord with this viewpoint. In this species a more lasting and severe hyperglycemia was developed by cortisone and glucagon treatment than ever previously established by hormonal administration. This was accompanied by marked glycogen infiltration of pancreatic duct epithelium and of β-cells. However, in no instance was islet destruction or permanent diabetes observed. However, the rabbit pancreas did show infrequent ballooned-out intraislet cells similar to those in the dog but without nuclear changes.

The present observations concerning pancreatic adaptation to diabetogenic stimuli are in agreement with those reported by others.[45-49] Most of these workers have suggested β-cell degranulation and hyperplasia, as well as neogenesis of β-cells, as the compensatory mechanisms. However, no unanimity of opinion as to origin of newformed β-cells exists.

In the embryo β-cells have been stated to develop either by differentiation from primitive ductular epithelium[50] or by transformation of acinar cells.[51] Under various conditions in postfetal life new formation of β-cells has been claimed to occur from acinar cells and from other islet cell types, as well as from duct epithelium.[47,52-55] The main evidence advanced for the former types of transformation has been either geographic contiguity or the supposed presence of different types of intracellular granules in the same cell or an acinar arrangement of cells.[47,52,53] However, it is generally acknowledged that, although primitive cell types may differentiate in various directions, the mature cell only reproduces itself. In our studies new formation of β-cells from hyperplastic duct epithelium via primitive intermediates could be traced, but no evidence of β-cell neogenesis from acinar cells, α-cells, or δ-cells was found. This is in agreement with the observations of others that apparent acinoinsular transformation or the interchangeability of various islet-cell types is an artifact due either to superimposition of two different cell types or to a confusion of mitochrondria with intracytoplasmic secretory granules.[50] Furthermore, the acinar arrangement of β-cells is also in accord with the idea that these cells are derived through transformation of ductal epithelium, and this has so been suggested by some.[56]

An additional finding which strongly supports these conclusions is the observation of the presence in the pancreas of the severely diabetic rabbit of two different categories of small intralobular ductules. One type showed marked vacuolation due to glycogen infiltration, while the other type was hyperplastic and nonvacuolated. It is from ductules of this latter type, which are thought to correspond with the primitive cell cords described in the adult guinea pig,[57] that β-cell neogenesis was observed to occur.

An interesting phenomenon, which has also previously been described[22,45] and for which no explanation exists, is the fact that hyperplasia of preexisting β-cells, as indicated by mitotic activity, only occurs for approximately the first ten days of treatment with diabetogenic hormones.

The problem of the stimulus for the increased β-cell functional activity and neogenesis still exists. There is a large weight of evidence which suggests that hyperglycemia per se will cause islet hyperplasia.[5,49,55,58] On the other hand, the possibility exists that there is a direct trophic action of cortisone on ductular epithelium. This latter possibility is supported by experiments showing that minimal doses of cortisone will produce islet hyperplasia and will cure alloxan diabetes in rats.[59] The present experiments do not clarify this point. However, they seem to support the

idea that hyperglycemia is the stimulus, since the extent of β-cell hyperplasia seemed to be significantly greater in the cortisone-plus-glucagon-treated animals which had the greatest severity and duration of hyperglycemia.

Summary

It has been shown in the rabbit treated with cortisone alone or simultaneously with glucagon that on any one hormone dosage the degree of hyperglycemia waned as treatment was prolonged. On the other hand, in the 50% partially pancreatectomized adult dog treated with 3 mg. of growth hormone per kilogram daily hyperglycemia is maintained with eventual β-cell destruction and production of permanent diabetes. The adaptation of the rabbit to diabetogenic hormones is attributed to increased insulin release from β-cells, as evidenced in the early stages by degranulation and mitoses of β-cells. In addition, there is new β-cell formation from small nonvacuolated hyperplastic ductules during long-continued treatment with a diabetogenic agent. These hyperplastic ductules are thought to be analogous to the primitive cell cords of the guinea pig and could be differentiated from other ductular epithelium, which in severely diabetic animals was vacuolated and contained glycogen. The dog, on the other hand, showed no neogenesis but rather destruction of β-cells. This was evidenced in early stages by scattered intrainsular ballooned-out cells containing neither Schiff-positive nor sudanophilic material but which showed nuclear pyknosis indicative of loss of cell viability. In later stages there was a loss of insular tissue, the residual islets consisting mostly of α-cells. An additional finding was the presence of both Schiff-positive and sudanophilic material in vacuolated duct epithelium frequently within the same cell.

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News and Comment

PERSONAL

Dr. E. T. Bell Gives Lecture.—The third Annual Carl V. Weller Lecture was given at the University of Michigan on Dec. 13, 1958, by Dr. E. T. Bell, of the University of Minnesota. Dr. Bell talked on the subject "The Clinical Course and the Pathological Anatomy of Diabetes Mellitus."

Dr. Shields Warren Addresses Group.—Dr. Shields Warren, of Boston, gave the dedicatory address on Dec. 16, 1958, of the Medical Research Center at Brookhaven National Laboratory.

Books

Treatment of Cancer and Allied Diseases: Vol. 1. Principles of Treatment. Second edition. Edited by George T. Pack, M.D., F.A.C.S., and Irving M. Ariel, M.D., F.A.C.S. Price, $22.50. Pp. 646, with 505 illustrations. Paul B. Hoeber, Inc. (medical book department of Harper & Brothers), 49 E. 33d St., New York 16, 1958.

This is a well-organized and well-illustrated volume. The editors have selected leading authorities in the various fields. Basic principles of the various phases of cancer management are covered in a broad fashion. Later volumes, eight in number, will cover specific categories of cancer. The book is very readable, and the illustrations are excellent and complete. Some of the charts are overcomplex and require careful study by the reader for complete understanding.

Almost one-half of the material deals with radiation and its relation to cancer. Considerable treatment detail is given. Specific surgical techniques are not described and will undoubtedly appear later.

Any physician who has to deal with cancer patients will find much valuable information in this volume.

Diagnostic Bacteriology. Fifth edition. Isabelle G. Schaub, A.B.; M. Kathleen Foley, M.A.; Elvyn G. Scott, M.T. (ASCP), and W. Robert Bailey, Ph.D. Price, $4.75. Pp. 338, with 35 tables and 14 illustrations. The C. V. Mosby Company, 3207 Washington Blvd., St. Louis, 1958.

"Diagnostic Bacteriology" can be recommended as a practical handbook for daily use in the clinical bacteriology laboratory. It is beyond doubt the best of its kind that this reviewer has seen. The authors are to be congratulated on their success both in remaining within the scope of the title they have chosen for their work and in the clear, concise manner in which they have set forth the laboratory procedures which they recommend. This must have been a difficult labor, since clinical bacteriology is an area in which proficiency is gained only by experience.

Of course some of the methods described will not meet with universal approval, but the book makes no pretense of being an encyclopedic work. There is a single sentence in the book which makes the whole work worth while, "However, the Quelling test remains the most rapid and satisfactory method for the identification of an organism as a pneumococcus, and is the only method available for the identification of pneumococci directly from clinical material."

The new section on mycotic infections is a most valuable addition to this fifth edition, even though this section consists of only twenty-four pages. It is a happy fact that in this section aspergilli are mentioned and are not passed off as mere laboratory contaminants.

The book is not intended as a general textbook of microbiology and will not replace such textbooks. Every laboratory where there is interest in diagnostic bacteriology should have this work on hand.

Autopsy Diagnosis and Technic. Fourth edition. Otto Saphir, M.D. Price, $8.50. Pp. 549, with 80 illustrations. Paul B. Hoeber, Inc. (medical book department of Harper & Brothers), 49 E. 33d St., New York 16, 1958.

Significant additions to the fourth edition of this useful autopsy manual include chapters dealing with autopsy tissue banking techniques and with autopsies performed on bodies of patients treated with radioactive isotopes. A short history of the autopsy, written by Dr. Sidney Farber, provides an interesting introduction. The sections on unexpected death and legal examinations, congenital cardiac anomalies, breast diseases, and bone and joint disease have been expanded. Especially useful are detailed descriptions of methods for removing tissues that are infrequently examined (such as nasopharynx and inner ear).

A few inconsistencies remain even in this edition. Some diagrams show the skin incision extending from the manubrium to the pubis, while others illustrate the Y-incision, which is strongly recommended in the test. The goal of providing a concise yet complete outline of autopsy technique and diagnosis has on the whole been met well.

The Guinea Pig in Research: Biology-Nutrition-Physiology. Mary Elizabeth Reid, Ph.D. Price, $2. Pp. 87, with 7 halftone plates and 12 tables. Human Factors Research Bureau, Inc., National Press Building, Washington 4, D. C., 1958.

The title of this booklet is somewhat misleading, since emphasis is placed on the use of the guinea pig in nutritional research. The known dietary needs of the guinea pig are described in detail. The rather extensive bibliography emphasizes nutrition. Physiology is considered largely in terms of growth and digestive functions. Brief mention is made of uses of guinea pig in microbiological, immunological, pharmacological, and radiological research. A listing of the places of production of some recognized strains of guinea pigs is helpful, as are the tables of growth rates and normal blood values.

Metabolism of Lipids. British Medical Bulletin 14:197-278 (Sept.) 1958. Price, $3.25. Medical Department, The British Council, 65 Davies St., London W1.

"Metabolism of Lipids" brings together, in a splendid collection of articles, the current status of knowledge in a broad area of lipid metabolism. The scope of the articles includes such topics as biosynthesis of fatty acids and cholesterol, a fat and sterol absorption and metabolism, hormonal effects on lipogenesis in mammary tissues, hormonal control of circulating lipids, the disorders associated with fat absorption, the effect of dietary fats on blood lipids, their relation to ischemic heart disease, removal of lipids from the bloom stream, and the essential fatty acids in relation to the skin and to human disease.

The authors have reviewed the literature, cited recent work, including their own studies, stated many problems as yet unsolved in the various areas discussed, and carefully pointed out the reservations and cautions to be observed in drawing conclusions based on current knowledge.

The introduction, on lipid metabolism is an excellent summary of the various articles and effectively relates the major points of interest in each to the subject at large.

These articles may be classed as required reading for workers in chemistry, biochemistry, physiology, and medicine involving fats or lipids. They will be welcomed as a rich source of information and as an excellent résumé of the current status of knowledge and of problems in the field.

Poisoning: A Guide to Clinical Diagnosis and Treatment. By W. F. von Oettingen, M.D. Price, $12.50. Pp. 627. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1958.

This is a useful text which covers a large variety of poisons. It is an attempt to survey the literature and to present the reports and conclusions in an abbreviated and useful form for the practitioner of medicine. The largest amount of space (394 pages) is devoted to the symptoms and treatment of a wide variety of poisoning and toxic-drug reactions. Long lists of symptoms and toxic substances which produce them are provided, which lead in some cases to tables containing over one hundred agents. This attempt to be comprehensive, while valuable, is not altogether successful. It is difficult to understand why strychnine is mentioned as an analeptic in barbiturate poisoning and why the special case of aspirin poisoning in children is omitted. Careful reading of the material on diagnosis and management should assist the practitioner in providing rational treatment where neglect of the principles of management may result in tragedy.

Etiology and Treatment of Leukemia. Walter J. Burdette, Ph.D., M.D., F.A.C.S. Price, $4. Pp. 167, with 13 illustrations. The C. V. Mosby Company, 3207 Washington Blvd., St. Louis 3, 1958.

This fine little book is a result of the First Louisiana Cancer Conference, which was sponsored by the Louisiana Division of the American Cancer Society. With the exception of Morwena Till, from the Chester Beatty Research Institute, of London, the participants were all outstanding American leukemia investigators. In this volume there are five articles on experimental animal leukemias, one article on diagnosis, and five articles on therapy. The articles on etiology are generally brief summaries of papers that these authors have previously published, whereas the papers on treatment are essentially review articles. Most aspects of therapy are considered to some extent, and this reviewer found Dr. Gellhorn's article on the

screening of antileukemic compounds particularly informative. The last chapter, by Dr. Burdette, is an excellent summary of present-day knowledge and concepts about the etiology and therapy of leukemia.

Generally, there is nothing new in this book, but for one who does not keep up with the progress in leukemia this can be a valuable source of current information. This reviewer found the discussion following each paper by the participants to be the most interesting part of the book. An extensive bibliography of three hundred eighty references is exceptionally valuable.

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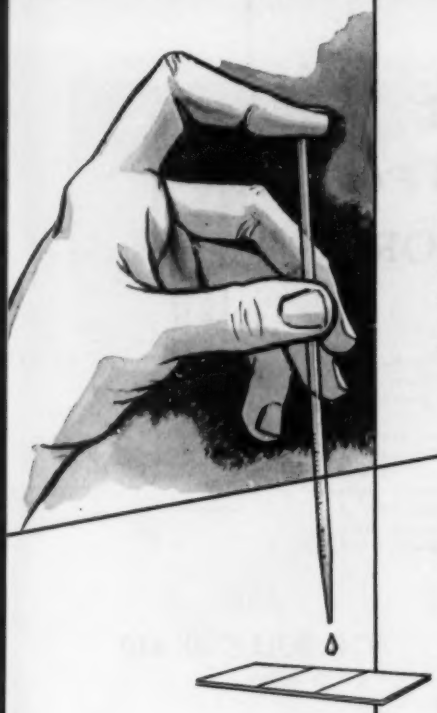

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